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*Research Article*

# Effects of combined oral contraceptive (Duofem) on some physiological parameters in female Wistar rats

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**Keywords:**
*Combined Oral Contraceptives, Physiological Parameters, Wistar rats*
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**ABSTRACT**

**Background:** Numerous **s**tudies have reported a relationship between oral contraceptives use and cardiovascular disease, altered levels of coagulation factors and thrombosis. The goal of this study was to examine the effect of Combined oral contraceptive (COC, Duofem) on some physiological parameters and elucidate possible mechanism of action in female wistar rats. **Methods**: Forty (40) female wistar rats aged 10-12 weeks weighing 180-250 g were used for the study. They were divided into four groups (A-D) of 10 rats each comprising 5 treated and 5 control rats. The treated rats received (by intragastric administration), 0.6mg/kg body weight of COC for 36, 48, 60 and 72 days in five-day cycles (four-days treatment with one-day break). The COC was given in 5-day cycles (4-day treatment with 1-day break). An autoanalyzer was used to perform a complete blood count (CBC or FBC). Enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of Protein C and S Antigen in citrated rat plasma. Antithrombin (AT) was determined by Chromogenic Assays. Prothrombin time (PT), Activated partial thromboplastin time (APTT) were performed using Sysmex CA-6000 Coagulation Analyzer. Serum electrolytes were determined using Audicom AC99000 and liver function tests by ELISA method. Erythropoietin was determined using rat EPO ELISA kit. Fibrinogen was estimated by Clauss Assay. **Results**: There were significant decreases in haemoglobin (Hb), packed cell volume (PCV), Red blood cell (RBC) count, white blood cell (WBC) counts, lymphocytes (L), prothrombin time (PT) concentration, fibrinogen (Fib) concentration, antithrombin (AT) concentration , protein C (PC), protein S (PS), interleukin-6 (IL-6) and interleukin-11 (IL-11) in all treated groups compared to controls ($P< 0.05$, respectively); in contrast, activities of alkaline phosphate (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) as well as sodium and potassium were increased. There were no significant changes in APTT, mean platelet volume (MPV), EPO concentration, MCH, MCV, MCHC, Neutrophil, Eosinophil, Basophil, Bicarbonate and Chloride in all treated groups compared to controls. Most of the changes in the Physiological parameters were observed in long term exposure group C and group D. **Conclusion:** Long term used of combined oral contraceptives may lead to more complications than short time use. COC users should be monitored for some physiological parameters.



## Introduction

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, 1977). Oral contraceptives prevent pregnancy primarily by inhibiting ovulation

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through the combined actions of progestin and estrogen. Progestin inhibits ovulation by suppressing the cyclical release of luteinizing hormone (LH) from the anterior pituitary gland. Progestin also creates thick cervical mucus that slows sperm transport and inhibits capacitation (the activation enzymes that permit the sperm to penetrate the ovum). Estrogen in contraceptives contributes to ovulation inhibition by suppressing the release of follicle-stimulating hormone (FSH) and LH (Campton, 2001).

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 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 and venous thromboembolism risk (Margolis *et al*, 2007).

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.

## Materials and Methods

### *Drugs*

The combined oral contraceptive used is DUOFEM®. 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 ethinyl estradiol 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 Ethinglestradiol and each brown tablet contains 75mg ferrous fumarate. DUOFEM® has a molecular weight of 312.4458g/mol (Chitturi et al, 2003).

Forty (40) female wistar rats aged 10-12 weeks weighing 180-250 g were used for the study. They were divided into four groups of 10 rats each comprising 5

treated and 5 control rats. The treated rats received 0.6mg/kg body weight of COC intragastically using oral gavage syringe for 36, 48, 60 and 72 days in five-day cycles (four-days treatment with one-day break). The COC was given intragastically 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. Experimental animals in the study were treated in accordance with the National Protection Laws of Animal Welfare (Akinsanya *et al*, 2010). Ethical clearance was obtained from the Ahmadu Bello University Animal Ethical Committee.

Other reagents and chemicals used were of analytical grade and of the purest quality available commercially. Animals were sacrificed after anaesthesia with chloroform and 5ml of blood was obtained via cardiac puncture and placed in plain and EDTA bottles. A haematology analyzer was used to perform a complete blood count (CBC or FBC). An enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of Protein C and S Antigen in citrated rat plasma. Antithrombin (AT) was determined by Chromogenic Assays. Prothrombin time (PT), Activated partial thromboplastin time (APTT) were performed using Sysmex CA-6000 Coagulation Analyzer. Serum electrolytes determined using Audicom AC99000. Interleukin 11 and 6 (IL-11, IL-6), erythropoietin (EPO) and liver function tests were determined by ELISA method. Fibrinogen was estimated by Clauss Assay (Clauss, 1957).

*Statistical Analysis*

The result obtained from this study was analyzed using SPSS version 20 for windows. Analysis of Variance (ANOVA) was used to compare means, and values were compared at $P < 0.05$. Post Hoc multiple comparisons for significant differences between groups were established by Turkey's HSD. All the data are expressed as Mean ± Standard Error of Mean (SEM).

**RESULTS**

The effects of COC (DUOFEM) on Haematological and Biochemical parameters in female wistar rats were obtained in this study. There were significant decrease in haemoglobin (Hb) packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) counts, lymphocytes (L), prothrombin time (PT), fibrinogen (Fib), antithrombin (AT), protein C (PC), protein S (PS), interleukin-6 (IL-6) and interleukin-11 (IL-11) in all treated groups compared to controls ($P< 0.05$). There was significant increase in alkaline phosphate (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium and potassium in all

**Table 1:** Effects of Combined Oral Contraceptives on Some Haematological Parameters in Female Rats

| Groups | Hb (g/dl) | PCV (%) | RBC ($\times10^{12}$/L) | WBC ($\times10^{9}$/L) | Platelet ($\times10^{9}$/L) |
|---|---|---|---|---|---|
| **A (36 Days)** | 12.3±1.2* | 38.5±2.1* | 6.1±07* | 6.1±1.3* | 783.6±13.9 |
| **Control** | 15.5±0.6 | 48.7±1.0 | 7.9±0.6 | 9.5±0.8 | 821.4±11.4 |
| **B (48 Days)** | 12.1±1.2* | 36.0±2.0* | 6.0±0.8* | 5.3±1.6* | 559.0±12.6 |
| **Control** | 14.6±0.7 | 43.6±1.5 | 7.3±0.4 | 8.5±1.1 | 691.4±11.0 |
| **C (60 Days)** | 13.0±0.9* | 40.4±1.5* | 6.6±0.7 | 6.7±1.8 | 508.2±11.1 |
| **Control** | 13.5±0.8 | 42.8±1.6 | 6.6±0.6 | 5.8±0.6 | 629.2±12.9 |
| **D (72 Days)** | 11.5±0.8* | 34.2±1.4 | 5.8±0.9 | 7.2±1.4* | 660.6±10.8 |
| **Control** | 13.6±0.6 | 40.9±0.9 | 6.8±0.7 | 9.7±1.0 | 642.2±10.5 |

*- significant ($P<0.05$).

**Table 2:** The Effects of Combined Oral Contraceptives on Some haematological Parameters in Female Wistar Rats

| Groups | N (%) | L (%) | M (%) | E (%) | B (%) | MPV (fl) | MCV (fl) | MCH (pg) | MCHC (g/dl) |
|---|---|---|---|---|---|---|---|---|---|
| **A (36 Days)** | 15.6±2.0* | 76.4±2.4* | 4.6±1.2 | 3.0±1.1 | 0.1±0.1 | 6.4±0.6 | 58.5±1.4* | 19.0±0.7* | 32.4±0.6* |
| **Control** | 9.6±1.2 | 83.6±1.8 | 3.2±1.1 | 3.4±1.2 | 0.2±0.1 | 6.5±0.4 | 70.8±1.7 | 19.5±0.8 | 31.5±0.7 |
| **B (48 Days)** | 16.8±2.6* | 70.4±2.7 | 9.0±1.4* | 3.2±1.3 | 0.3±0.1 | 6.6±0.4 | 59.0±1.6 | 20.1±0.9 | 33.8±1.0 |
| **Control** | 14.8±2.0 | 76.8±1.5 | 4.8±1.6 | 3.2±1.1 | 0.3±0.1 | 6.4±0.4 | 59.1±1.4 | 20.4±0.9 | 33.9±0.7 |
| **C (60 Days)** | 10.2±1.2* | 83.6±1.6 | 3.8±1.1 | 2.0±0.8* | 0.2±0.1 | 6.8±0.7 | 61.0±1.5 | 19.7±0.6 | 32.4±0.7 |
| **Control** | 17.0±2.8 | 75.2±2.9 | 4.2±1.1 | 3.2±1.1 | 0.3±0.1 | 6.6±0.6 | 64.5±1.3 | 20.3±0.8 | 31.5±0.7 |
| **D (72 Days)** | 14.8±2.1* | 73.4±2.2 | 5.8±1.3 | 3.4±1.0 | 0.1±0.1 | 6.2±0.5 | 59.6±1.4 | 18.8±0.5 | 31.7±0.8 |
| **Control** | 12.0±2.0 | 78.2±1.7 | 5.4±1.3 | 3.8±1.1 | 0.2±0.1 | 6.3±0.7 | 61.6±1.2 | 18.8±0.8 | 33.0±1.0 |

*- significant ($P<0.05$). Neutrophils and monocytes were lowest for treated group C (60 Days), while Lymphocytes, Eosinophil, MPV, MCH and MCHC were lowest in treated group D (72 Days).

**Table 3:** The Effects of Combined Oral Contraceptives on some Haemostatic Parameters and Cytokines in Female Wistar Rats

| Groups | PT (sec) | APTT (sec) | FIB (mg/mL) | IL-11 (ng/L) | IL-6 (ng/L) | EPO (iu/L) |
|---|---|---|---|---|---|---|
| **A (36 Days)** | 10.7±0.9* | 20.1±1.3 | 96.4±2.4* | 45.2±7.6 | 30.9±7.2* | 12.3±1.9 |
| **Control** | 13.1±0.7 | 21.1±0.7 | 140.8±2.9 | 42.4±9.6 | 20.8±1.2 | 10.5±1.2 |
| **B (48 Days)** | 9.5±1.1* | 17.7±1.4 | 91.6±3.1* | 51.1±9.1 | 30.7±8.7 | 14.4±1.5 |
| **Control** | 12.9±0.5 | 17.6±1.2 | 127.4±3.8 | 50.1±9.1 | 26.0±1.6 | 11.9±1.1 |
| **C (60 Days)** | 9.5.**0**±0.7* | 19.5±1.2 | 92.0±2.9* | 37.9±1.3* | 34.7±8.0* | 10.8±1.3 |
| **Control** | 13.6±1.0 | 20.8±1.4 | 111.8±3.4 | 41.7±1.0 | 37.7±1.8 | 11.1±1.4 |
| **D (72 Days)** | 10.9±0.9* | 21.2±0.7 | 86.4±2.2* | 46.8±1.8* | 26.1±1.5* | 10.7±1.2 |
| **Control** | 13.5±1.0 | 22.1±1.0 | 114.2±3.3 | 49.0±2.2 | 47.1±9.8 | 12.4±1.2 |

*-significant (P<0.05). The lowest values of PT, IL-6 and Fibrinogen were observed in the treated group D (72 Days).

treated groups compared to controls (P< 0.05). There were however no significant changes in activated partial thromboplastin time (APTT), mean platelet volume (MPV), erythropoietin (EPO), MCH, MCV, MCHC, Neutrophil, Eosinophil, Basophil, Bicarbonate and Chloride in all treated groups compared to controls.

**Table 4:** The Effects of Combined Oral Contraceptives on Some Anticoagulants in Female Wistar Rats

| GROUPS | AT (u/ml) | PC (u/ml) | PS (u/ml) |
|---|---|---|---|
| **A (36 Days)** | 0.55±0.03* | 0.64±0.05* | 0.68±0.06* |
| **Control** | 0.62±0.06 | 0.75±0.08 | 0.82±0.09 |
| **B (48 Days)** | 0.48±0.05* | 0.52±0.06* | 0.67±0.04* |
| **Control** | 0.68±0.04 | 0.72±0.04 | 0.74±0.06 |
| **C (60 Days)** | 0.46±0.07* | 0.56±0.03* | 0.58±0.03* |
| **Control** | 0.68±0.06 | 0.74±0.08 | 0.78±0.05 |
| **D (72 Days)** | 0.43±0.06* | 0.48±0.05* | 0.58±0.07* |
| **Control** | 0.72±0.08 | 0.76±0.07 | 0.81±0.08 |

*-significant (P<0.05). The lowest values of AT, PC and PS were observed in treated group D (72 Days).

Most of the changes were observed in long term exposure to COC. The histology of the kidney shows no morphological changes in both treated groups and controls. Changes were however observed in the morphology of hepatocytes in the liver which was infiltrated with fats in groups C and D.

**Table 5:** The Effects of Combined Oral Contraceptives on Some Liver Enzymes in Female Wistar Rats

| GROUPS | ALP (u/L) | ALT (u/L) | AST (u/L) |
|---|---|---|---|
| **A (36days)** | 117.2±4.3* | 79.4±2.9* | 137.0±5.3* |
| **B (48days)** | 116.8±3.0* | 117.2±2.9* | 220.8±8.1* |
| **Control** | 72.3±6.1 | 74.4±3.9 | 117.2±5.2 |
| **C (60days)** | 120.6±3.7* | 86.6±2.4* | 231.8±8.1* |
| **Control** | 82.0±4.2 | 63.2±3.4 | 151.0±4.3 |
| **D (72days)** | 122.8±2.9* | 99.8±3.5* | 272.2±3.5* |
| **Control** | 88.2±4.1 | 65.8±2.8 | 145.2±4.0 |

*- Significant (P<0.05). The highest values of ALP, ALT and AST were observed in treated group D (72 Days)

## DISCUSSION

In this study the effect of COC on some Physiological parameters in female wistar rats was investigated. The result shows a reduction in haemoglobin(Hb), Packed cell volume(PCV), Red blood cell (RBC), White blood cell (WBC) counts and lymphocytes in all the groups that were given COC, compared to the controls (P<0-05 respectively).

**Table 6:** The Effects of Combined Oral Contraceptives on some Serum Electrolytes in Female Wistar Rats

| GROUPS | ALP (u/L) | ALT (u/L) | AST (u/L) |
|---|---|---|---|
| **A (36days)** | 117.2±4.3* | 79.4±2.9* | 137.0±5.3* |
| **B (48days)** | 116.8±3.0* | 117.2±2.9* | 220.8±8.1* |
| **Control** | 72.3±6.1 | 74.4±3.9 | 117.2±5.2 |
| **C (60days)** | 120.6±3.7* | 86.6±2.4* | 231.8±8.1* |
| **Control** | 82.0±4.2 | 63.2±3.4 | 151.0±4.3 |
| **D (72days)** | 122.8±2.9* | 99.8±3.5* | 272.2±3.5* |
| **Control** | 88.2±4.1 | 65.8±2.8 | 145.2±4.0 |

*-Significant (P<0.05)

The finding of lower Hb, PCV, RBC, WBC and Lymphocytes agrees with the finding of Sajida *et al.* (2006). The finding is however contrary to that of other investigators like Bulur *et al.* (2006), Babatunde *et al.* (2003) and Abdalla (2008), who reported no changes in full blood count in women on COC. The least values of Hb, PCV and RBC were found in group D. It could be that the use of COC for a longer period may lead to anaemia. The finding of lower Hb, PCV and RBC counts in COC treated groups compared to controls may be as a result of the haemodilautoary effect of estrogen and the effect of estrogen on the cytokines like interleukin-11 and interleukin -6. The findings of lower values of WBC counts in COC treated groups suggest that prolonged use of COC may make the user susceptible to nonspecific infection and thus altered immune response.

The findings of lower WBC count in COC treated groups was in agreement with that of Sajida *et al.* (2006), and contrary to the findings of Surasak *et al.* (2007) who observed no changes in WBC count and that of Araz *et al.* (2009) who observed increase in WBC count in COC treated female wistar rats. 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. There were no

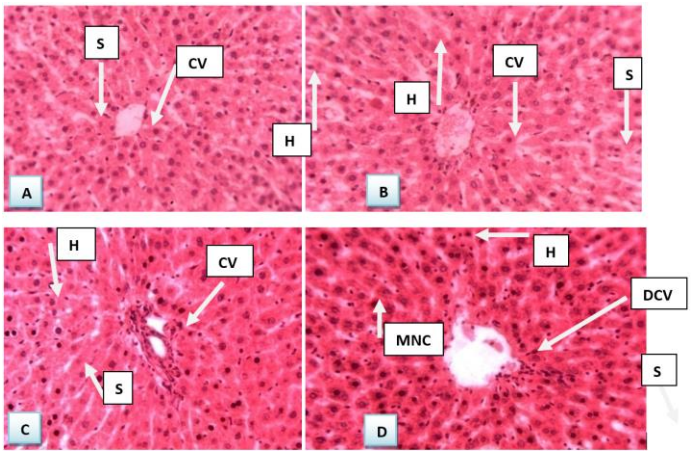


Plate 1: Photomicrographs of experimental groups - Liver sections; Shows Transverse Sections of Liver. Group A and B Showed Normal Liver Architecture with the Central Vein (CV), Sinusoids (S) and Hepatocytes (H) Appearing Normal. Mononuclear Cells (MNC) were Noted in Group C and D Respectively and Dilated Central Vein (DCV) was Observed in Group D (×250) H & E

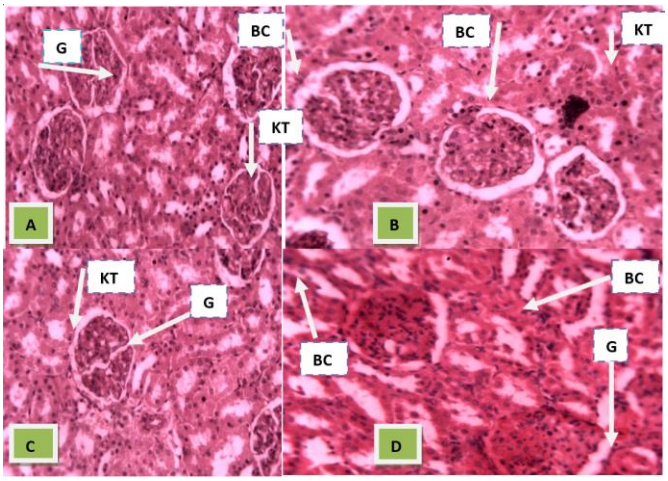


Plate 2: Photomicrographs of experimental groups - Kidney sections. Shows Normal Architectures of Longitudinal Sections of kidney. The Bowman's Capsules (BC), Glomerulus (G), and the Kidney Tubules (KT ) of the Kidney Sections in Group A, B, C and D all Appeared Normal (×250) H&E

significant changes observed in COC treated groups in platelet counts and mean platelet volume (MPV) compared to the controls. This finding agrees with that of Surasak *et al.* (2007), Peter (2013), Bulur *et al.* (2006) and Babatunde *et al.* (2003). The observation was however contrary to that of Sajida *et al.* (2006) who reported significant increase on MPV in subjects on COC. There were no significant changes in MCV, MCH, Neutrophil, Eosinophil and Basophil counts in COC treated groups compared to the controls. This finding is in agreement with that of Peter (2013), Bulur

*et al.* (2006) and Babatunde *et al.* (2013), and contrary to the findings of Sajida *et al.* (2006). In general, the use of COC in female wistar rats suppressed haemopoiesis in this study resulting in lowering of Hb, PCV, RBC and WBC counts.

There was no significant change in serum erythropoietin level in all compared with 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 decrease fibrinogen levels in all the COC treated groups compared to the control. This finding differs from that of Eliana *et al.* (2014) and Peter *et al.* (2013) who reported increase in fibrinogen level in women taking COC. The finding is also contrary to that of Akhigbe *et al.* (2008) who found no significant change in fibrinogen level in female wistar rats treated with COC. The depletion of fibrinogen observed in the present study might result in coagulopathy.

The association between increase in plasma fibrinogen and thrombosis and the risk of myocardial infarction are well established. Higher level of fibrinogen raises the risk of stroke. Damage to the liver may be the cause of deficiency in fibrinogen level by a third generation COC (DUOFEM).This may reduce risk of thrombosis in users. Fibrinogen functions as a messenger molecule that coordinates and regulates the body response to inflammation. Low fibrinogen levels are associated with low risk of cardiovascular diseases. Prothrombin Time (PT) significantly reduce in all the COC treated groups compare to the controls ($P<0.001$). There was no significant reduction in Activated Prothrombin Time (APTT). This finding agrees with that of Abdalla *et al.* (2008) in Sudan found significant reduction in PT and APTT in women taking COC. Babatunde *et al.* (2008) found that there was no significant change in the level of APTT in Nigerian women taking COC for three months. This is in agreement with the finding of this study.

Also, Eliana *et al.* (2014) found reduced PT and APTT in Albanian women taking COC. Reduction in prothrombin time may be as a result of reduced serum level of fibrinogen. Estrogen is also said to increase coagulation factors leading to decreased prothrombin time. The finding of this study is also in agreement with that of Ahmed *et al.* (2008) and Nasir *et al.* (2008) who reported a significant reduction in APTT and PT in women taking COC. The COC (DUOFEM) used in this study is likely to cause prothrombotic effect. This may lead to hypercoagulability and thrombosis.

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 estroblast's synthesis of IL-6 receptors (Jean *et al.*, 1992). IL-11 and IL-6 are haemopoiesis-promoting factors capable of enhancing the growth of myloid, erythroid and megakaryiocytic progenitor cells. They are capable of mediating a complex array of pro- and anti-inflammatory effects. Reduction in IL-11 and IL-6 may be responsible for decreased RBC, PCV, WBC and platelet counts in this study. 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 pleotropic 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. Interleukin 6 (IL-6), promptly and transiently produced in response to infections and tissue injuries, contributes to host defence through the stimulation of acute phase responses, hematopoiesis, and immune reactions. IL-6 has been demonstrated to have a pivotal role in the pathogenesis of rheumatoid arthritis, Castleman's disease and Crohn's disease exemplified by the use of an anti-IL-6 biological therapy. However, IL-6 is also associated with the autoimmune disease systemic sclerosis and has been shown to be directly fibrotic (Rachon *et al.*2002).

There was a significant decrease in serum level of antithrombin (AT), protein C (PC) and protein S (PS) in COC treated groups compared to the controls. Proteins C and S and antithrombin are components of the anticoagulant system. In haemostasis, the procoagulant system is in balance with the anticoagulant and fibrinolytic system (Karl *et al.*, 2008). A decrease in AT, PC and PS may cause thrombosis. This finding is in agreement with that of Soare (2010) and John *et al.* (2012) who observed lower values of PC in women using oral contraceptives. Decreased AT, PC and PS may be responsible for the changes observed in haemostasis. AT, PC and PS are vitamin K-dependent. Damage to the liver may be responsible for the reduction of serum level of AT, PC and PS in COC treated groups.

There were increased values of alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in all COC treated groups compared to controls (P<0.001). The liver plays a central role in the metabolism of estrogens and progesterones. COC acts directly or indirectly on the liver to produce a variety of biological effects which have both physiological and pathological significance (Trussel, 2007). Higher levels of liver enzymes in the blood streams are prime indicators of liver damage. The use of COCs has been rarely associated with liver tumours, both benign (hepaticadeomas and nodular hyperplasia) and malignant (hepatocellular carcinoma). The finding of increased liver enzymes is in agreement with that of Dickerson *et al.* (1992) and contrary to that of Surasak *et al.* (2007) who found no changes in liver enzymes in women on COC.

Raised serum level of ALP, AST and ALT in treated groups may be due to functional alterations involving the hepatic excretory mechanism. There was a significant increase in serum sodium level in group D ( P<0.044) compared to the control. There was no significant increase in serum sodium level in treated groups A (36 days), B and C compared to the controls. Potassium was significantly increased in groups C (60 days) and D Sodium retention has been reported in women taking COC (Akhigbe *et al.*, 2008). The result in this study is contrary to that of Taneepanichskul *et al.* (2007) who reported no significant changes in electrolytes in women taking COC.

Hypertension occasionally occurs in women receiving estrogen-progesterone combination for contraception. Estrogen raises the plasma level of renin and increases the production rate of aldosterone which leads to water and sodium retention (Nicholas *et al.*, 1974; Kang *et al.*, 2011), reported reduced potassium level and increased sodium level in female rats. There was no significant change in bicarbonate and chloride in treated groups compared to the controls. Body electrolyte balances are critical for normal cellular function and maintaining adequate blood and plasma volume and osmolality.

## CONCLUSION

COCs have evolved from one generation to another. The COC (DUOFEM) used in this study is a third generation COC. DUOFEM causes changes in Haematological and Biochemical parameters by decreasing the level of Hb, PCV, WBC, IL6 &11, Fibrinogen, Antithrombin, Protein C & S, and increasing the level of liver enzymes and serum electrolytes. The histology of the kidney shows no morphological changes in both treated groups and controls. Changes were however observed in the morphology of hepatocytes in the liver which was infiltrated with fats in groups C and D.

We recommend further study at the gene level devoted to COC (DUOFEM) effect on coagulation to clarify the mechanism by which the hormones influence the expression of circulating proteins in haemostasis. There is need for an effective, risk-free combined oral contraceptive. COC users should be monitored for some physiological parameters.

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