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INVESTIGATION INTO THE EFFECTS OF ORAL CONTRACEPTIVES ON SOME HAEMATOLOGICAL PARAMETERS OF WISTAR ALBINO RAT *RATTUS RATTUS*

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ABSTRACT

In this study, the in vivo effect of oral contraceptives namely Microgynon a combined pill (0.15mg levonorgestrel and 0.03mg ethinylestradiol) and Primolut -N a mini pill (5mg norethisterone) onsome haematological parameters of wistar albino rat were investigated. The haemoglobin count, packed cell volume, total white blood cell count, platelet count, neutrophil and lymphocyte count were assayed. Test results showed that the oral contraceptives had an increasingeffect on the haematological parameters. This increase was not dose dependent. The highest increase of 18.00 ± 1.40 vs control 14.30 ± 1.10 (g/dl) was obtained for Hb at the concentration of 0.72µg at 2 hours duration (p< 0.05).The blood PCV, WBC, platelet, neutrophil and lymphocyte analysis also showed increases. The possible implications of these findings on the female rat and by extrapolation on the female 'homo sapiens' are that laboratory tests involving full blood analysis should be undergone before these drugs are taken. Check-up tests should also be performed every six months, to identify women who might be at risk of developing minor or serious side effects that might affect major organs of the body.

Key words: Blood, ethinylestradiol, haematological parameters, lenonorgestrel, norethisterone, oral contraceptives.

INTRODUCTION

Haematological studies is of great importance to man. They are also of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment (Addas *et al*., 2012). Haematological parameters are used as good indicators of the physiological status of animals (Addas *et al*., 2012; Aderemi, 2004). The major functions of the white blood cell and its differentials are to protect the body by fighting infections, defending the body by phagocytosis against invasion by foreign organisms and to produce or transport and distribute antibodies in immune response (Forlan *et al*., 1999).Therefore, animals with low white blood cells are at high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (Agaje and Uko, 1998).

Oral contraceptives (OCs) are drugs taken orally for the prevention of pregnancy. Nowadays, OCs are widely available and used for family planning. It is estimated that the drugs are now used by more than

100 million women throughout the world(Kuhl and Goethe, 1990; CHPE, 1984; Bremner, et al 1977; Kay *et al.*, 1974). The Population Reference Bureau (2005) reported that 12% of Nigerian married women are on contraceptives out of which 8% use modern methods of contraception. The National Cancer Institute (2003) stipulated that there are currently two types of OCs available. The most commonly prescribed OC contain two man made versions of the female hormones (estrogen and progesterone) that are similar to the hormones the ovaries normally produce. The second type of OC available is called the mini pill. It contains only a progestogen. The oral contraceptives: 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 for contraception and also for other non contraceptive benefits (Okoye *et al*., 2012; Briggs, 1980). These oral contraceptives have been known to have some side effects ranging from nausea to cancer (Mishel *et al*., 1976). 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, 1990). It is worthy to note that because of problems associated to oral contraceptives, the manufacturers of these oral drugs have continually decreased hormone levels in order to provide formulation with maximum efficiency and minimum side effects (Okoye *et al.*, 2011; Kaunitz, 2004; Grimes *et al*., 1993).

MATERIALS AND METHOD

Microgynon was purchased from Schering AG Germany.

Primolut- N was purchased from Medipharm (Pvt) Ltd., Lahore Licencee of Schering AG. Federal Republic of Germany.

A total of 108 female wistar albino rats *rattus rattus* (average weight 100.00 ± 10.00g) were used for the tests. These were obtained from the animal house of the Biochemistry Department, Faculty of Science, University of Port Harcourt. The rats were divided into two groups of 54 rats each for the different drugs. The drugs were administered orallywhile the initial weight of the drugs fed to the rats were scaled down to a ratio of the normal dosage taken by an average woman of 55kg. The animals were on their normal diets before the drug administration and were continued on this diet after that. Five doses of the contraceptive drugs (microgynon: 0.36, 0.72, 1.40, 1.80 and 3.60 µg per100g body weight and primolut –N: 10.00, 20.00, 40.00, 50.00 and100.00 µg per 100g body weight) were administered for each analysis. A set of 9 rats were used as controls for each drug analysis and no contraceptive drugs were administered to them. The tests were monitored for 24 hours intervals ranging from 2hours, 4 hours and 24 hours. 18 rats from each drug group were sacrificed after each time interval (3 rats from each dose group). This was done by cardiac puncture, with the animal under anesthesia (chloroform) in a desiccator. The blood collection was done immediately and were stored in EDTA sample containers.

Determination of the Effects of the Drugs on Full Blood Count

Measurement of Packed Cell Volume (PCV) by Centrifugation Method

The packed cell volume of the haematocrit (literally blood separation), is a measure of

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the relative mass of red cells present in a sample of whole blood. It is used as a measure of testing for anaemia, in conjunction with accurate estimation of haemoglogin (Dacie and Lewis 1975; Baker *et al*., 1985).

Well - mixed anticoagulated blood was allowed to enter the tube by capillarity, leaving at least 15mm unfilled. The tube was sealed by heating the dry end of the rube rapidly in a blue flame. After centrifugation for 10 minutes, the PCV was measured using a Hawskley micro Haematocrit reader (Dacie and Lewis 1975).

Determination of Hb Concentration

The Hb concentration was determined by dividing the value of the PCV by 3.

Hb concentration = PCV /3

Determination of White Blood Cell (WBC) Total Count

Quantitative and qualitative alterations in the circulating leucocytes characterize many diverse diseases and are often diagnostically significant. It is a routine part of all clinical evaluations (Linman, 1975).

Reagents used were glacial acetic acid, distilled water, methyl violet

Into 0.38ml of the diluting fluid in a test tube was washed 0.02ml of blood. This was mixed properly and used to fill the counting chamber which had been cleaned. The cells in the four corner square millimetre were counted using Naubeurs chamber (Baker *et al.,* 1985).

Determination of (WBC) Differentials

Blood smears were made on slides and were allowed to dry. Leishmans stain was used on them and allowed to stand for 2 minutes. They were then washed out with distilled water. The slides were placed in oil emersion and then viewed under the microscope at x100 magnification. Laboratory DC counter was then used for counting (Baker *et al* 1985).

RESULT

Statistical Analysis:

Data analysis was performed using the Statistical Package for the Social Sciences software (SPSS, version 11.0). Data is displayed in mean ±SD. The statistical method of one way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups. Differences were considered significant whenever the p-value is $p<0.05$.

Effect of Microgynon on Hb Concentration

The results of the effect of microgynon on Hb concentration are presented in the Table 1. The results showed that the drug did have an increase on the Hb concentration. The highest Hb concentration was obtained at the dose of 0.72µg/100g. The differences in dosage and the time were statistically significant on the effect of the drug on the Hb concentration levels at 95.0% confidence level ($p< 0.05$).

Table 1: Effect of microgynon on Hb concentration of wistar albino rat

Microgynon	Hb concentration (g/dl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	14.30±1.10	14.30±2.30	14.30±1.60
0.36	15.00±1.20	14.30±0.50	15.00±1.10
0.72	18.00±1.40*	18.30±1.60*	14.30±0.50
1.40	15.00±0.50	14.30±1.70	14.00±1.00
1.80	15.30±2.10	14.00±1.00	14.00±1.00
3.60	14.00±1.70	15.00±1.70	15.20±7.90

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Microgynon on PCV

The results of the effect of microgynon on PCV are represented in the Table 2. The results showed that the drug did have an increase on the PCV. The highest PCV was obtained at the dose of 0.72µg/100g. The differences in dosage and time were statistically significant on the effect of the drug on the PCV levels at 95.0% confidence level ($p < 0.05$).

Table 2: Effect of microgynon on PCV of wistar albino rat

Microgynon	PCV (%)		
µg/100g	2hrs	4hrs	24hrs
0.00	43.00±3.51	43.00±6.92	43.00±5.13
0.36	45.00±3.70	43.00±1.52	45.00±3.51
0.72	54.00±4.04*	55.00±5.03*	43.00±1.52
1.40	45.00±1.52	43.00±5.19	42.00±3.00
1.80	46.00±6.35	42.00±3.05	42.00±3.00
3.60	42.00±5.13	45.00±5.19	46.00±6.08

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect on Microgynon on WBC

The results of the effect of microgynon on WBC are presented in the Table 3. The results showed that the drug did have an increase on the WBC. The highest WBC count of 5400.00 ± 321.45 was obtained at 24 hours with the dose of 0.36 µg/100g. The differences in dosage and time were statistically significant on the effect of the drug on the WBC levels at 95.0% confidence level ($p < 0.05$).

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Table 3: Effect of microgynon on WBC of wistar albino rat

Microgynon	WBC (cells/ µl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	4800.00±321.45	4800.00±230.94	4800.00±321.45
0.36	4300.00±288.67	5200.00±208.16*	5400.00±321.45*
0.72	5100.00±173.20*	5100.00±173.20	4900.00±57.73
1.40	4300.00±472.58	5200.00±57.73*	4900.00±173.20
1.80	4800.00±57.73	4900.00±152.75	4900.00±152.75
3.60	4900.00±57.73	4800.00±230.94	4800.00±208.16

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Microgynon on WBC Differentials (neutrophils)

The results of the effect of microgynon on neutrophils are presented in the Table 4. The results showed that the drug did have an increase on the neutrophils. The differences in dosage and time were statistically significant on the effect of the drug on the neutrophils levels at 95.0% confidence level ($p < 0.05$).

Table 4: Effect of microgynon on WBC differentials (neutrophils) of wistar albino rat

Microgynon	NEUTROPHILS(cells/µl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	49.00±1.00	49.00±0.57	48.00±1.15
0.36	48.00±0.50	48.00±1.00	48.00±1.00
0.72	49.00±0.50	49.00±0.57	50.00±0.57*
1.40	48.00±2.51	50.00±0.00*	50.00±1.52*
1.80	49.00±0.00	50.00±1.52*	50.00±1.52*
3.60	50.00±0.57*	49.00±1.15	49.00±1.15

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Microgynon on WBC Differentials (lymphocytes)

The results of the effect of mcirogynon on lymphocytes are presented in the Table 5. The results showed that the drug did have an increase on the lymphocytes. The differences in dosage and time were statistically significant on the effect of the drug on the lymphocytes levels at 95.0% confidence level ($p < 0.05$).

Table 5: Effect of microgynon on WBC differentials (lymphocytes) of wistar albino rat

Microgynon	LYMPHOCYTES(cells/µl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	45.00±2.60	45.00±1.70	45.00±0.50
0.36	46.00±0.50	48.00±1.50*	46.00±0.50
0.72	48.00±1.70*	48.00±1.70*	46.00±0.50
1.40	46.00±2.00	46.00±0.50	46.00±0.00
1.80	45.00±2.30	46.00±2.00	46.00±2.00
3.60	50.00±3.60*	45.00±1.15	45.00±2.00

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Microgynon on WBC Differentials (eosinophils)

The results of the effect of microgynon on eosinophils are presented in the Table 6. The results showed that the drug did not have much significant effect on the eosinophils. However there appear to be a decrease in the eosinophils with increasing drug dose. The differences in dosage and time were statistically significant on the effect of the drug on the eosinophils level at 95.0% confidence level ($p < 0.05$).

Table 6: Effect of microgynon on WBC differentials (eosinophils) of wistar albino rat

Microgynon	EOSINOPHILS(cells/µl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	2.00±1.00	2.00±0.57	2.00±1.00
0.36	1.00±0.57	2.00±0.57	2.00±0.57
0.72	1.00±0.57	1.00±0.57	1.00±0.57
1.40	2.00±0.57	1.00±1.00	1.00±0.57
1.80	2.00±0.57	1.00±0.57	1.00±0.57
3.60	1.00±0.57*	2.00±0.57*	2.00±0.57

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Microgynon on WBC Differentials (monocytes)

The results of the effect of microgynon on monocytes are presented in the Table 7. The results showed that the drug did not have much significant effect on the monocytes. However there appear to be an increase in the monocytes. The differences in dosage and time were statistically significant on the effect of the drug on the monocytes levels at 95.0% confidence level ($p < 0.05$).

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Table 7: Effect of microgynon on WBC differentials (monocytes) of wistar albino rat

Microgynon	MONOCYTES(cells/µl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	2.00±0.57	2.00±0.57	2.00±0.57
0.36	3.00±0.57	3.00±1.00	3.00±0.57
0.72	3.00±0.57	3.00±0.57	3.00±0.57
1.40	3.00±0.57	4.00±0.57	3.00±0.57
1.80	4.00±1.73*	3.00±0.57	3.00±0.57
3.60	3.00±1.52	4.00±0.57	4.00±1.52*

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p< 0.05$).

Effect of Primolut-N on Hb Concentration

The results of the effect of primolut –N on Hb concentration are presented in the Table 8. The results showed that the drug did have an increase on the Hb concentration. The highest Hb concentration was obtained at the dose of 20µg/100g. The differences in dosage and time were statistically significant o the effect of the drug on the Hb concentration levels at 95.0% confidence level, ($p< 0.05$).

Table 8: Efeect of primolut –N on Hb concentration of wistar albino rat

Primolut-N	Hb concentration (g/dl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	13.00±2.30	14.30±1.60	14.00±1.10
10.00	15.30±0.50*	15.00±1.10	14.00±1.20
20.00	15.20±1.60*	16.30±0.50*	15.00±1.40
40.00	15.00±1.70	15.00±1.00	15.00±0.50
50.00	15.00±0.00	15.00±1.00	15.00±2.10
100.00	15.00±1.70	15.00±7.90	15.00±1.70

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p< 0.05$).

Effect of Primolut-N on PCV

The results of the effect of primolut-N on PCV are presented in the Table 9. The results showed that the drug did have an increase on the PCV. The highest PCV was obtained at the dose 20µg/100g. The differences in dosage and time were statistically significant on the effect of the drug on the PCV levels at 95.0% confidence level ($p< 0.05$).

Table 9: Effect of primolut-N on PCV of wistar albino rat

Primolut-N	PCV (%)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	39.00±6.92	43.00±5.13	42.00±3.51
10.00	46.00±1.52*	45.00±3.51	42.00±3.70
20.00	46.00±5.03*	49.00±1.52*	45.00±4.04
40.00	45.00±5.19	45.00±3.00	45.00±1.52
50.00	45.00±3.05	45.00±3.00	45.00±6.35
100.00	45.00±5.19	45.00±6.08	45.00±5.13

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Primolut-N on WBC

The results of the effect of primolut-N on WBC are presented in the Table 10. The results showed that the drug did have an increase on the WBC. The highest WBC count of 5300.00 ± 173.20 was obtained at 4hours with the dose of 40.00µg/100g. The differences in dosage and time were statistically significant on the effect of the drug on the WBC levels at 95.0% confidence level ($p < 0.05$).

Table 10: Effect of primolut-N on WBC of wistar albino rat

Primolut-N	WBC (cells/µl)		
µg/100g	2hrs	4hrs	24hrs
0.00	4300.00±230.94	4800.00±321.45	4900.00±321.45
10.00	4800.00±208.16	4800.00±321.45	4900.00±288.67
20.00	4800.00±173.20	4800.00±57.73	4800.00±173.20
40.00	5000.00±57.73	5300.00±173.20*	5200.00±472.58*
50.00	4900.00±152.75	5200.00±152.75*	5000.00±57.73
100.00	4900.00±230.94	4800.00±208.16	5200.00±57.73

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Primolut-N on WBC Differentials (neutrophils)

The results of the effect of primolut-N on neutrophils are presented in the Table 11. The results showed that the drug did have an increase on the neutrophils. The differences in dosage and time were statistically significant on the effect of the drug on the neutrophils levels at 95.0% confidence level ($p < 0.05$).

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Table 11: Effect of primolut-N on WBC differentials (neutrophils) of wistar albino rat

Primolut-N	NEUTROPHILS (cells/µl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	48.00±0.57	48.00±1.15	50.00±1.00
10.00	49.00±1.00	49.00±1.00	50.00±0.50
20.00	49.00±0.57	48.00±0.57	49.00±0.50
40.00	53.00±0.00*	50.00±1.52	51.00±2.51*
50.00	49.00±1.52	51.00±1.52*	53.00±0.00*
100.00	49.00±1.15	49.00±1.15	51.00±-0.57

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Primolut-N on WBC Differentials (lymphocytes)

The results of the effect of primolut-N on lymphocytes are presented in the Table 12. The results showed that the drug did have an increase on the lymphocytes. The differences in dosage and time were statistically significant on the effect of the drug on the lymphocytes level at 95.0% confidence level ($p < 0.05$).

Table 12: Effect of primolut-N on WBC differentials (lymphocytes) of wistar albino rat

Primolut-N	LYMPHOCYTES (cells/ µl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	43.00 ±1.70	45.00 ±0.50	45.00 ±2.60
10.00	45.00 ±1.50	45.00 ±0.50	46.00 ±0.50
20.00	45.00 ±1.70	45.00 ±0.50	45.00 ±1.70
40.00	46.00 ±0.50	45.00 ±2.00	47.00 ±2.00
50.00	49.00 ±2.00*	47.00 ±2.00	43.00 ±2.30
100.00	49.00 ±1.15*	45.00 ±2.00	47.00 ±2.60*

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Primolut-N on WBC Differentials (eosinophils)

The results of the effect of primolut-N on eosinophils are presented in the Table 13. The results showed that the drug did not have much significant effect on the eosinophils. However there appear to be a decrease in the eosinophils. The differences in dosage and time were statistically significant on the effect of the drug on the eosinophils levels at 95.0% confidence level ($p < 0.05$).

Table 13: Effect of Primolut-N on WBC differentials (eosinophils) of wistar albino rat

Primolut-N	EOSINOPHILS (cells/ µl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	3.00±0.57	3.00±1.00	3.00±1.00
10.00	2.00±0.57	2.00±0.57	1.00±0.57
20.00	2.00±0.57*	2.00±0.57*	2.00±0.57*
40.00	2.00±1.00	2.00±0.57	1.00±0.57
50.00	1.00±0.57	1.00±0.57	2.00±0.57
100.00	1.00±0.57	2.00±0.57	1.00±0.57

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

Effect of Primolut-N on WBC Differentials (monocytes)

The results of the effect of primolut-N on monocytes are presented in the Table 14 below. The results showed that the drug did not have much significant effect on the monocytes. However there appear to be an increase in monocytes. The differences in dosage and time were statistically significant on the effect of the drug on the monocytes levels at 95.0% confidence level ($p < 0.05$).

Table 14: Effect of primolut-N on WBC differentials (monocytes) of wistar albino rat

Primolut-N	MONOCYTES (cells/µl)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	2.00±0.57	2.00±0.57	2.00±0.57
10.00	4.00±1.00	4.00±0.57	3.00±0.57
20.00	4.00±0.57*	4.00±0.57*	4.00±0.57*
40.00	2.00±0.57	3.00±0.57	3.00±0.57
50.00	3.00±0.57	3.00±0.57	2.00±1.73
100.00	3.00±0.57	4.00±1.52	3.00±1.52

Results are means of three determinations ± standard deviation

*Statistically significant at 95.0% confidence level. ($p < 0.05$).

DISCUSSION

The results of some of the haematological parameters studied on are shown on Tables - 1 to 14. The results showed that the oral contraceptives increased the haematological parameters investigated. However the increases were not of major significance. This result is similar to the results obtained by Godsland *et al*., (1983) who compared the haematological indices between women of four ethnic groups and the effect of oral contraceptives. The report stated that the effect of oral contraceptive on haematological indices was analysed but not found to be significant. However, oral contraceptives produces increase in serum

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iron in patients with hepatitis (Adebamowo and Adekunle, 1999).

A deficient iron intake could account for high incidence of low haemoglobin (Godsland, *et al*., 1983). However, Ghoneim, *et al*., (1975) reported that women who were slightly anaemic before beginning the contraceptive had their haemoglobin and haematocrit showing significant increase especially among women who had taken the drug for more than four years. Fisch and Freedman (1973) reporting from the Kaiser-Permanente contraceptive study, analysed the incidence of anaemia in 1,083 oral contraceptive users and 2,653 non users. In order to exclude the effects of menstrual loss, they standardized the results for this important variable. Since all the subjects were of similar high socio – economic status, the likelihood that they were taking an iron deficient diet was small.

The residual differences between the oral contraceptive users and non-users could then be attributed to the direct metabolic action of the sex steroids. The authors demonstrated slight but significantly lower haemoglobin levels in pill users, together with a lower red cell count and an increase in the mean size of the red cells. While there is evidence that oral contraceptives have complex reaction on iron metabolism and red cell formation, the predominant clinical effect is a reduced incidence of iron deficiency anaemia due to diminished menstrual loss (Fisch and Freedman 1973).

The body must have iron to make haemoglobin and to help transfer oxygen to the muscle. If the body is low in iron, all body cells, particularly muscles in adults and brain cells in children, do not function up to par. Haemoglobin provides the main transport of oxygen and carbon in the blood. It is composed of "globin", a group of amino acids that form a protein and "heme", which contains iron. It is an important determinant of anaemia (decreased haemoglobin) or poor diet/nutrition or malabsorption. Haematocrit is the measurement of anaemia, dehydration or possible overhydration. PCV measures the average size of the red blood cells and their volume. These components together can indicate iron deficiency anaemia. B12/folate deficiency anaemia, of rheumatoid arthritis. White blood cell count measures the total number of white blood cell in a given volume of blood. Since WBC's kill bacteria, this count is a measure is the body's response to infection(Okoye, 2008; Ugwueme, 2011; Khan and Zafar, 2005; Adebamowo and Adekunle, 1999).

From the results of this study, it was observed that oral contraceptives generally increased haematological parameters. Therefore, full medical laboratory tests should be undergone before prescription of these drugs. The tests should include full blood analysis. There should be check-up tests every six months.

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