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INVESTIGATION INTO THE EFFECTS OF ETHINYLESTRADIOL, LEVONORGESTREL AND NORETHISTERONE ON DIRECT AND TOTAL BILIRUBIN OF WISTAR ALBINO RATS (*RATTUS RATTUS)*

N. F. Okoye and A. A. Uwakwe

Department of Biochemistry, University of Port Harcourt, Nigeria
P.M.B 5323, Port Harcourt Nigeria.

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ABSTRACT

Some oral contraceptives namely Microgynon a combined pill (0.15mg levonorgestrel and 0.03mg ethinylestradiol), Primolut -N a mini pill (5mg norethisterone) and Postinor a post coital pill (0.75mg levonorgestrel)were analysed for their in vivo effects on rat plasma direct and total bilirubin. The in vivo effects of the oral contraceptives on rat plasma direct bilirubin showed that the drugs increased the direct bilirubin levels. The effect of the drugs on the directbilirubin levels were also time dependent and dose dependent with the highest increase obtained at 24 hours duration while the lowest increase occurred at 2 hours.Microgynon showed the highest increase (6.88 ± 0.00 vs control 3.19 ± 0.00 µmol/l) ($p< 0.05$) followed by Primolut–N (4.92 ± 0.00 vs control3.19 ± 0.00 µmol/l). The in-vivo effects of the oral contraceptives on rat plasma total bilirubin showed that the drugs increased the total bilirubin levelsin a concentration dependent manner. The effect of the drugs on the total bilirubin were also time dependent with the highest increase obtained at 24 hours while the lowest increase occurred at 2 hours duration. Microgynon showed the highest increase (18.31 ± 0.00 vs control 7.40 ± 1.85 µmol/l). It was observed from this study that most women do not undergo liver function test as part of their medical examination before these medicines are prescribed to them. Since these drugs increased the bilirubin levels, it is suggested that a thorough medical checkup that includes liver function tests be undergone before taking these oral contraceptives. It is also recommended that medical examination be done every twenty four weeks.

Key words: Bilirubin, contraceptive, ethinylestradiol, levonorgestrel, norethisterone

INTRODUCTION

Contraceptive is regarded as a drug, device or other means of preventing pregnancy. In this day and age, contraception is a vital factor in many homes and in the lives of many women, giving users a private, self directed means with which to control fertility and plan their family. Oral contraceptives (OCs) are drugs taken orally for the prevention of pregnancy. 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). Therefore it is important that the efficacy of these contraceptives are effectively monitored. Also the effect of these drugs on the liver and other organs of the body need to be analysed from time to time to ensure that they are safe to be taken

by women who are healthy otherwise. The oral contraceptives ; Microgynon a combined pill (0.15mg levonorgestrel and 0.03mg ethinylestradiol), Primolut- N a mini pill (5mg norethisterone) and Postinor a post coital pill (0.75mg levonorgestrel) are among the most common drugs used in Nigeria in particular and the world at large for contraception and also for other non contraceptive benefits (Okoye *et al*., 2008; Briggs, 1980). These oral contraceptives have been known to have some side effects ranging from nausea to cancer (Mishell*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). However, manufacturers of these oral contraceptives 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). Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. An increase in bilirubin concentration in the serum or tissues is called jaundice (Sherlock, 1951).

MATERIALS AND METHOD

Microgynon was obtained from Schering AG Germany.

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

Postinor was obatined from Chemical works of Gedeon Richter Ltd. Budapest, Hungary.

Reagent kits were bought from Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co., Antrim, United Kingdom BT29 4QY

A total of 162 female Wistar albino rats *rattusrattus* (average weight 100.00 ± 10.00g) were used for the tests. These were obtained from the animal house of the Department of Biochemistry, Faculty of Science, University of Port Harcourt. The rats were divided into three groups of 54 rats each for the different drugs. The drugs were administered orally, 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 (standard commercial feed) 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, primolut –N: 10.00, 20.00, 40.00, 50.00 and100.00 µg per 100g body weight and postinor: 1.50, 3.00, 6.00, 7.50, 15.00 µg per100g body weight) were administered for each analysis. A set of nine 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. After each time interval, 18 rats from each drug group were sacrificed (three 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 the blood stored in lithium heparin sample containers. The blood was centrifuged at 3000 rotations per minute for 3 minutes and the plasma used for the analysis.

Determination of the effects of the drugs on bilirubin level

Bilirubin levels were determined by colorimetric method as outlined in the Randox kits used, based on the method

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described by Jendrassik and Grof (1938). In this method, direct conjugated bilirubin reacts with diazotisedsulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin was determined by the reaction with diazotisedsulphanilicacid.

Statistical analysis:
Data analysis was performed using the Statistical Product and Service Solutions Software (SPSS, version 11.0). Data was displayed as mean ±SD. One way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups. Differences were considered significant at the p-value is $p<0.05$.

RESULTS

***In vivo* effect of microgynon on rat plasma direct bilirubin**

The effect of microgynon on plasma direct bilirubin are presented in the Table 1. The drug had a progressive increase on direct bilirubin levels. The increase was dose dependent. The highest increase of 6.88 µmol/l was observed for the highest dose of 3.60µg/100g body wt. Dosage of the drug had a statistically significant effect ($p < 0.05$) on the plasma direct bilirubin levels. Differences in time were not statistically significant ($p > 0.05$) on the effect of the drug on the plasma direct bilirubin levels.

Table 1: *In vivo* effect of microgynon on rat plasma direct bilirubin expressed in µmol/l

Microgynon	Plasma Direct Bilirubin (µmol/l)		
µg/100g body wt	2hr	4hr	24hr
0.00	3.19±0.27	3.19±0.00	3.19±0.27
0.36	3.69±0.00*	3.69±0.27	2.70±0.27
0.72	5.17±0.27*	5.17±0.00*	5.41±0.27*
1.40	5.41±0.00*	5.41±0.00*	5.65±0.27*
1.80	6.15±0.27*	6.15±0.00*	6.39±0.00*
3.60	6.64±0.27*	6.64±0.00*	6.88±0.00*

Results are means of three determinations ± standard deviation
*Statistically significant at 95.0 confidence level.

***In vivo* effect of primolut –N on rat plasma direct bilirubin**

There was no increase observed until the drug dose was raised to 40 mg /100 g body weight. The highest increase of 4.92 µmol/l was observed for the highest dose of 100.00µg/100g body weight of the drug primolut N. The differences in dosage from 40 mg /100 g body weight were statistically significant ($p < 0.05$) on the plasma direct bilirubin levels. The differences in time were not statistically significant ($p > 0.05$) on the effect of the drug on plasma direct bilirubin levels(Table 2).

Table 2: *In vivo* effect of primolut –N on rat plasma direct bilirubin expressed in µmol/l

Primolut	Plasma Direct Bilirubin (µmol/l)		
µg/100g body wt	2hr	4hr	24hr
0.00	3.19±0.00	3.19±0.27	3.19±0.27
10.00	3.19±0.00	3.19±0.27	3.19±0.27
20.00	3.19±0.27	3.19±0.00	3.19±0.00
40.00	3.69±0.27*	3.69±0.00*	3.69±0.27*
50.00	3.69±0.00*	4.43±0.27*	3.69±0.00*
100.00	4.92±0.00*	4.92±0.00*	4.92±0.00*

Results are means of three determinations ± standard deviation
*Statistically significant at 95.0% confidence level.

***In vivo* effect of postinor on rat plasma direct bilirubin**

The effect of postinor on plasma direct bilirubin are presented in Table 3. There were no increases observed until the drug dose was raised to 7.50mg / 100 g body weight for 4 hr and 24 hr and for all intervals at 15.00 mg / 100 g body weight. The differences in time were not statistically significant ($p > 0.05$) on the effect of the drug on the plasma direct bilirubin levels.

Table 3: *In vivo* effect of postinor on rat plasma direct bilirubin expressed in (µmol/l)

Postinor	Plasma Direct Bilirubin (µmol/l)		
µg/100g body wt	2hr	4hr	24hr
0.00	3.19±0.27	3.19±0.27	3.19±0.27
1.50	3.19±0.27	3.19±0.27	3.19±0.27
3.00	3.19±0.27	3.19±0.27	3.19±0.27
6.00	3.19±0.27	3.19±0.27	3.19±0.27
7.50	3.19±0.00	3.40±0.27*	3.40±0.27*
15.00	3.40±0.27*	3.40±0.00*	3.40±0.27*

Results are means of three determinations ± standard deviation
*Statistically significant at 95.0% confidence level.

***In vivo* effect of microgynon on rat plasma total bilirubin**

Microgynonhad a progressive increase on total bilirubin levels. The increase was dose dependent. The highest increase of 18.31±0.00µmol/l was observed for the highest dose of 3.60µg/100g body wt. The differences in dosage were statistically significant ($p < 0.05$) on the effect of the drug on the plasma total bilirubin levels. The differences in time were not statistically significant ($p > 0.05$) on the effect of the drug on the plasma total bilirubin levels (Table 4).

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Table 4: *In vivo* effect of microgynon on rat plasma total bilirubin expressed in µmol/l

Microgynon	Plasma Total Bilirubin (µmol/l)		
µg/100g body wt	2hr	4hr	24hr
0.00	7.40±1.85	7.40±0.00	7.40±1.85
0.36	14.80±1.85*	15.17±0.00*	15.72±0.00*
0.72	16.64±1.85*	16.83±0.00*	17.02±1.85*
1.40	17.20±1.85*	17.20±0.00*	17.76±1.85*
1.80	17.76±0.00*	17.76±1.85*	17.94±1.85*
3.60	18.13±0.00*	18.13±1.85*	18.31±0.00*

Results are means of three determinations ± standard deviation
*Statistically significant at 95.0% confidence level.

***In vivo* effect of primolut –N on rat plasma total bilirubin**

Primolut – N had a progressive increase on total bilirubin levels. The highest increase of 14.80±1.85µmol/was observed for the highest dose of 100.00µg/100g body wt. The differences in dosage were statistically significant ($p < 0.05$) on the effect of the drug on the plasma total bilirubin levels. The differences in time were not statistically significant ($p > 0.05$) on the effect of the drug on the plasma total bilirubin levels.

Table 5: *In vivo* effect of primolut –N on rat plasma total bilirubin expressed in µmol/l

Primolut –N	Plasma Total Bilirubin (µmol/l)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	7.40±1.85	7.40±1.85	7.40±1.85
10.00	11.10±1.85	11.28±0.00	11.47±0.00
20.00	11.10±0.00*	11.10±1.85	12.00±0.00
40.00	12.00±0.00*	12.90±0.00*	12.00±0.00*
50.00	12.00±0.00*	12.90±1.85*	13.87±0.00*
100.00	12.90±1.85*	12.90±1.85*	14.80±1.85*

Results are means of three determinations ± standard deviation
*Statistically significant at 95.0% confidence level.

***In vivo* effect of postinor on rat plasma total bilirubin**

The effect of postinor on plasma total bilirubin are presented in Table 6. The drug had a progressive increase on total bilirubin levels. The increase was dose dependent. The highest increase of 12.95±0.00µmol/was observed for the highest dose of 15.00µg/100g body wt. The differences in dosage were statistically significant ($p < 0.05$) on the effect of the drug on the plasma total bilirubin levels. The differences in time were not statistically significant ($p > 0.05$) on the effect of the drug on the plasma total bilirubin levels.

Table 6: *In vivo* effect of postinor on rat plasma total bilirubin expressed in µmol/l

Postinor	Plasma Total Bilirubin (µmol/l)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00	7.40±1.85	7.40±1.85	7.40±1.85
1.50	9.25±1.85*	9.62±0.00	9.25±1.85
3.00	9.25±1.85*	9.8±0.18	9.62±0.18
6.00	10.17±0.00*	10.17±0.18*	10.54±0.18*
7.50	11.10±1.85*	11.28±0.18*	11.47±0.18*
15.00	12.02±0.00*	12.02±0.18*	12.95±0.00*

Results are means of three determinations ± standard deviation
*Statistically significant at 95.0% confidence level.

DISCUSSION

The results showed that the drugs increased plasma direct and total bilirubin levels with mirogynon having the highest (direct: 6.88 ± 0.00 vs control 3.19 ± 0.27, total: 18.31 ± 0.00 vs control 7.40 ± 1.85 µmol/l) followed by primolut-N (direct: 4.92 ± 0.00 vs control 3.19 ± 0.27, total: 14.80 ± 1.85 vs control 7.40 ± 1.85 µmol/l) and then postinor (direct: 3.40 ± 0.00 vs control 3.19 ± 0.27, total: 12.95 ± 0.00 vs control 7.40 ±1.85 µmol/l). Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. In the liver, bilirubin is conjugated with glucuronic acid to form a soluble compound. This conjugated bilirubin passes down the bile duct and is excreted into the gastrointestinal tract. An unconjugated, albumin bound form is also present in the circulation. It is insoluble and does not normally pass through the kidneys into the urine (Ashafa*et al*., 2012; Hild*et al*., 2010 and Akanji*et al*., 2008). An increase in bilirubin concentration in the serum or tissues is called jaundice. Jaundice occurs in toxic or infectious diseases in the liver e.g. hepatitis B or obstruction of the bile duct in rhesus incompatible babies (Pratt and Kaplan, 2000).

Useful information may be obtained by determining which form of bilirubin is elevated. High levels of conjugated or direct bilirubin indicate in the bile duct of gall bladder. Unconjugated or indirect bilirubin indicates that too much haemoglobin is being destroyed or that the liver is not actively treating the haemoglobin it is receiving. Oral contraceptives can cause jaundice but this is rare (Ashafa*et al*., 2012). Earlier publication by Okoye*et al.*, (2012) indicated that the *in vivo* effects of the oral contraceptives on rat plasma ALT showed that the drugs also elevated the activity of this enzyme in a concentration and time dependent manner. The effect of the drugs on the enzyme were also time dependent with the highest activation obtained at 24 hours while the least activation occurred at 2 hours. A recent work by Ekhator*et al*., (2014) using rabbits showed that oral contraceptives containing low dose synthetic hormones still affected liver function.The drugs are contraindicated where there is a history of recurrent intrahepatic cholestasis of pregnancy and acute or chronic disturbance of liver function which can be congenital or acquired (Ekhator*etal* 2014; Uwakwe*et al.*, 2012; Okoye 2008). It is not yet known whether the estrogenic or progestogenic

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components of oral contraceptives cause the hepatic abnormalities. The available data suggest that neither oestrogens nor progestogens in low doses impair hepatic excretory processes (WHO 2010; Hargreaves, 1969).

This study has shown that the drugs raised the direct and total bilirubin levels, therefore, care must be taken during prescription and tests must be run occasionally, to check women at risk of developing jaundice.

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