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A STUDY OF THE EFFECTS OF ORAL CONTRACEPTIVES ON PLASMA UREA OF WISTAR ALBINO RAT *rattus rattus*

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ABSTRACT

Oral contraceptives such as Microgynon a combined pill (0.15mg levonorgestrel and 0.03mg ethinylestradiol) and Primolut -N a mini pill (5mg norethisterone) were investigated for their in-vivo effects on wistar albino rat *rattus rattus* plasma urea levels. Test results showed that the drugs had a lowering effect on plasma urea levels, in a concentration and time dependent manner. Microgynon showed the highest decrease of 70.83% at 24 hours duration and at the highest concentration level of 3.60µg/100g body wt (0.0007 ± 0.00 mmol/) while Primolut –N showed a decrease of 54.16% at the same time interval (0.0011 ± 0.00 mmol/l). The differences in weight and hour were statistically significant on the effect of the drug on the plasma urea levels at 95.0% confidence level (P<0.05). From this study, it was clear that the oral contraceptives decreased the wistar albino rat plasma urea levels. So for the women taking these drugs, it is imperative that full medical laboratory tests should be undergone before prescription of the drugs. The tests must include kidney function. There should also be check up tests every six months.

KEY WORDS: Microgynon, oral contraceptives, Primolut-N, urea.

INTRODUCTION

Oral contraceptives (OCs) are drugs taken orally for the prevention of pregnancy. Nowadays, OCs are widely available and used for family planning. OCs were subjected to the most rigorous tests before being made available for general use. From the results of these tests, it was possible to make fairly accurate predictions. Although, few substances have ever received as much careful investigation as have oral contraceptives, doubts still remain, perhaps this is because a high standard is demanded of a preparation that is to be taken by healthy women for many years (Kay et al 1974). Oral contraceptives first became available to women in the early 1960s. The convenience, effectiveness, and reversibility of action of these birth control pills (popularly known as “the pill”) have made them the most popular form of birth control (Henderson et al, 1991; Liu and Lebrun, 2006). 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, 1984). 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, 1990; Kaunitz, (2004). Henderson et al (1991) 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, 1990; Henderson et al, 1991; Smith et al, 2003). 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-releasingHormne (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.

Urea is a waste product derived from protein breakdown in the liver. Plasma normally contains 2.5 – 6.5 mmol of urea per litre. This represents the balance between its production and excretion. The blood urea rises if excretion is impaired. Some causes of slight to moderate rises in blood urea, not due to renal disease, are fluid depletion with haemoconcentration, excess tissue breakdown, haematesis and congestive cardiac failure causing circulatory impairment and mower glomerular filteration rate (Wootton and Freeman, 1982).

MATERIALS AND METHOD

Microgynon was bought from Schering AG Germany.

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

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The urea reagent kit was bought from Linear Chemicals S.L. (Cromatest) Joaquim Costa, 18, 2a planta. 08390 Montgat – Barcelona, Spain.

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 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 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 a lithium heparin sample containers. The blood was centrifuged at 3000 rotations per minute for 3 minutes and the blood plasma were separated and used for the analysis.

Urea determination

Urea levels were determined by Enzymatic colorimetric endpoint method. The Principle of this method is that Urea is hydrolysed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield a blue cromophore. The intensity of the colour formed is proportional to the concentration of urea in the sample (Young, D. S. 1995; Tietz, N. W 1995).

The Reagent kit contained reagent 1: (urease ≥500U/ml), stabilizers. Reagent 2 : (buffered chromogen), phosphate buffer (20mmol/l pH 6.9), EDTA (2 mmol/l), sodium salycilate (60 mmol/l), sodium nitroprusside (3.4mmol/l). Reagent 3: Alkaline hypochlorite,sodium hypochlorite (10 mmol/l), NaOH (150mmol/l), urea standard, urea (8.3 mmol/l). The working reagent was prepared by mixing 1 ml of reagent 1 with 24 ml of reagent 2.

1.00ml of the working reagent was mixed with 10µl of the sample. The standard tube contained 1.00ml of the working reagent and10µl of the standard. The blank tube had 1.00ml of working reagent. The mixture was incubated for 5 minutes at 37 ^{o}C and then 1.00ml reagent 3 was added to the tubes. The mixture was further incubated for 5 minutes at 37^{o}C and absorbance of the samples read against the reagent blank at 600nm with Spectronic -20 spectrophotometer

Calculations

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} \times C_{standard} = \text{mg/dl urea} \times 0.1665 = \text{mmol/l}$$

Normal values 2.5 – 6.6 mmol/l

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 $P<0.05$.

RESULTS AND DISCUSSION

The mean results ± SD of urea determination are presented in Tables 1 and 2 and illustrated in Figures1 and 2. Test results showed that the drugs had a lowering effect on plasma urea levels, in a concentration and time dependent manner. Microgynon showed the highest decrease at 24 hours duration (0.0007 ± 0.00 vs control 0.0024 ± 0.00 mmol/l) followed by Primolut (0.0011 ± 0.00 vs control 0.0024 ± 0.00 mmol/l). The highest decrease of 70.83% was observed at 24 hours interval for the highest dose of 3.6µg/100g body weight. The differences in weight and hour were statistically significant on the effect of the drug on the plasma urea levels at 95.0% confidence level ($p<0.05$). Blood Urea Nitrogen (BUN) is a waste product derived from protein breakdown in the liver. Increases can be caused by excessive protein intake, kidney damage, certain drugs, low fluid intake, intestinal bleeding, exercise, heart failure or decreased digestive enzyme production by the pancreas. Decreased levels are most commonly due to inadequate protein intake, malabsorption, or liver damage. Urea is the chief end product of protein metabolism in the body. The importance of the urea concentration in blood lies in its value as an indicator of kidney function. Azotemia (an abnormal increase in plasma urea levels) is seen mainly in renal disorders, dehydration, increase protein catabolism, high-protein diets, or gastrointestinal hemorrhage. There are two types of azotemia. The first, prerenal azotemia is caused by impaired perfusion of the kidneys due to decreased cardiac output or for any of the former causes. The second, postrenal azotemia, is caused by an obstruction in the urine outflow such as nephrolithiasis, prostatism, and tumors of the genitourinary tract. The clinical significance, of the urea level in plasma is usually determined in conjugation with the plasma creatinine level. In prerenal azotemia, an increase in the plasma urea level is usually associated with a normal plasma creatinine level, where as in the postrenal azotemia, there is an increase in both the urea and the plasma creatinine levels. A decrease in the plasma urea level may be associated with acute dehydration, malnutrition and pregnancy.

Table 1*: In vivo* effect of Microgynon on rat plasma urea expressed in mmol/l

Microgynon dose	Urea (mmol/l)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00 (control)	0.0024 ± 0.00	0.0024 ± 0.00	0.0024 ± 0.00
0.36	0.0022 ± 0.00[a]	0.002 ± 0.01[b]	0.0013 ± 0.00[c]
0.72	0.0019 ± 0.00[a]	0.0016 ± 0.00[b]	0.0011 ± 0.00[c]
1.40	0.0016 ± 0.00[a]	0.0012 ± 0.00[b]	0.0009 ± 0.00[c]
1.80	0.0014 ± 0.00[a]	0.0012 ± 0.00[b]	0.0008 ± 0.00[c]
3.60	0.0013 ± 0.00[a]	0.001 ± 0.00[b]	0.0007± 0.00[c]

Results are means of three determinations ± standard deviation
[abc].Different letters in a given row denote significant difference, P<0.05.

Figure 1: Effect of Microgynon on plasma urea (mmol/l)

Table 2: *In vivo* effect of Primolut-N on rat plasma urea expressed in mmol/l

Primolut -N	Urea (mmol/l)		
µg/100g body wt	2hrs	4hrs	24hrs
0.00 (control)	0.0024 ± 0.00	0.0024 ± 0.00	0.0024 ± 0.00
10.00	0.002 ± 0.00[a]	0.002 ± 0.00[a]	0.0019 ± 0.00[b]
20.00	0.0018 ± 0.00[a]	0.0018 ± 0.00[a]	0.0017± 0.00[b]
40.00	0.0017 ± 0.00[a]	0.0016 ± 0.00[b]	0.0015 ± 0.00[c]
50.00	0.0017 ± 0.00[a]	0.0016 ± 0.00[b]	0.0015 ± 0.00[b]
100.00	0.0013 ± 0.00[a]	0.0012 ± 0.00[b]	0.0011 ± 0.00[c]

Results are means of three determinations ± standard deviation
[abc].Different letters in a given row denote significant difference, P<0.05.

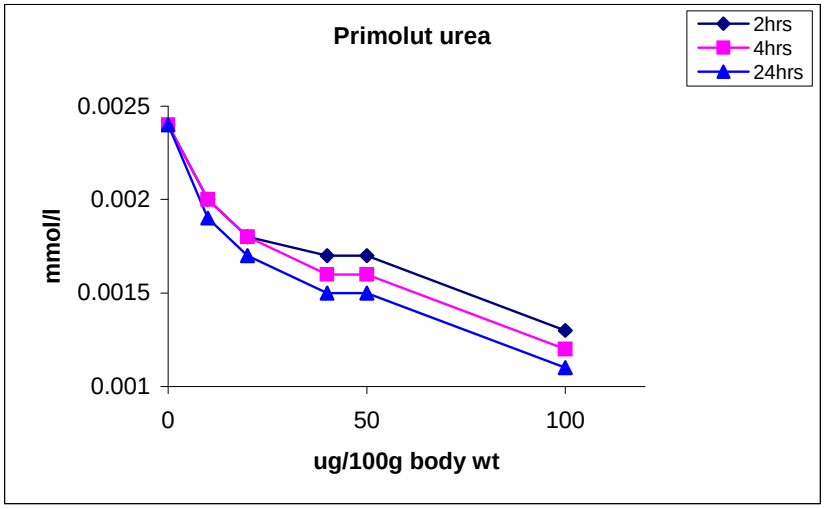

Figure 2: Effect of Primolut-N on plasma urea (mmol/l)

CONCLUSION

From this study, it was clear that the oral contraceptives decreased the rat plasma urea levels. So for the women taking these drugs, it is imperative that full medical laboratory tests should be undergone before prescription of the drugs. The tests must include kidney function. There should be check up tests every six months.

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