Effects of oral contraceptives - Microgynon and Primolut-N on plasma creatinine of wistar albino rat.

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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 Invivo effects on wistar albino rat plasma creatinine levels. Test results showed that the drugs raised plasma creatinine levels in a concentration dependent manner but the increase in creatinine levels were not time dependent. The highest increase of 300% was observed for Microgynon at the highest concentration of 3.60µg/100g body wt (354 ± 0.00 µmol/l). The highest increase obtained for Primolut-N was 182.75% (236 ± 29.50µmol/l). The differences in dosage were statistically significant on the effect of the drugs on the plasma creatinine levels at 95.0% confidence level (P<0.05). Creatinine is a protein breakdown product and its level is a reflection of the bodies muscle mass, but unlike urea, creatinine is almost independent of diet. Since the blood level reflects the balance between production and excretion, it varies little from day to day if renal function remains constant. This result makes it imperative that kidney function tests are needed for women before using these drugs.

Keywords: Oral contraceptives, Microgynon, Primolut-N, plasma creatinine

1. Introduction
Contraceptive is a drug, device or other means of contraception. Today, contraception is a vital factor in married life 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. Around 100 million women throughout the world now use the drugs. (Kay et al., 1974; Kuhl & Goethe, 1990; CHPE, 1984). 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 in Nigeria for contraception and also for other non contraceptive benefits. Like any other drug, they have some side effects ranging from nausea to cancer. 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 & Jespersen, 1990). Over the years however, hormone levels have continually decreased in order to provide formulation with maximum efficiency and minimum side effects (Vessey et al., 1982; Shelepova et al., 2005). Over the years however, hormone levels have continually decreased in order to provide formulation with maximum efficiency and minimum side effects (Grimes et al, 1993; Liu & Lebrun (2006).

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). During the past 30 yrs, many other formulations have been developed and marketed with steadily decreasing dosages of both estrogen and progestin components. All the formulations initially marketed after 1975 contain less of several progestins.

The use of these agents is associated with very low pregnancy rates, similar to those for formulations with higher doses of steroid (Vessey et al.,1982; Shelepova et al., 2005). Over the years however, hormone levels have continually decreased in order to provide formulation with maximum efficiency and minimum side effects (Grimes et al, 1993). The estrogen/progestin combination is the most effective type of OC formulation, because these preparations consistently inhibit the midcycle gonadotropin surge, and thus prevent ovulation. Such formulations also act on other aspects of the reproductive process. They alter the cervical mucus, making it thick, viscid and scanty, which retards sperm penetration. They also alter motility of the uterus and oviduct, thus impairing transport of both ova and sperm. Furthermore, they alter the endometrium so that its glandular production of glycogen is diminished and less energy is available for the blastocyst to survive in the uterine cavity.

Creatinine is a protein breakdown product and its level is a reflection of the bodies muscle mass. Creatinine is a metabolite of creatine. Production is endogenous, determined by muscle mass, and unlike urea is almost independent of diet. Since the blood level
reflects the balance between production and excretion, it varies little from day to day if renal function remains constant. In normal people, the serum or plasma concentration ranges from 55-125 µmol/litre. It increases in renal failure (Wootton & Freeman, 1982).

2. Materials and method

Microgynon was bought from Schering AG Germany. Primolut- N was bought from Medipharm (Pvt) Ltd., Lahore. Licencsee of Schering AG. Federal Republic of Germany. The creatinine reagent kit was bought from Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom BT29 4QY.

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 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: 1.00, 20.00, 40.00, 50.00 and100.00 µg per 100g body weight were administered for each analysis. A set of 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. A set of rats from each drug group were sacrificed after each time interval. This was done by cardiac puncture, with the animal under anesthesia (chloroform) in a desiccator. The blood collection was done immediately and was stored in a lithium heparin sample containers. The blood was centrifuged and the blood plasma were separated and used for the analysis.

2.1 Creatinine determination

Creatinine levels were determined by colorimetric method (with deproteinization). The principle of this method is that creatinine in alkaline solution reacts with picrate to form a coloured complex (Henry, 1974).

The Reagent kit contained solution 1: Standard (177µmol/l), solution 2: Picric acid (35 mmol/l), solution 3: Sodium hydroxide (1.6 mol/l), TA 651 Trichloroacetic acid (TCA) (1.2mol/l). The Working reagent was prepared by mixing 10ml of solution 2 and 10ml of solution 3.

The sample was first deproteinized by mixing 1.0ml of Trichloroacetic acid (TCA) and 1.0ml of sample. The mixture was vigorously stirred with a glass rod to evenly disperse the precipitate. The mixture was then centrifuged at 2500 rpm for 10 minutes the supernatant was then separated and used for the assay as listed below:

1.00 ml of the working reagent was mixed with 1.00ml of the supernatant. The standard tube contained 1.00ml of the working reagent, 0.5ml of TCA and 0.5 ml of solution 1. The blank tube had 1.00ml of working reagent, 0.5ml of TCA and 0.5ml of distilled water. The mixture was let to stand fro 20 minutes at 25°C and the absorbance of the sample and standard were read against the blank at 520nm with Spectronic -20 spectrophotometer.

Calculations

\[
\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 177 = \mu\text{mol}
\]

Normal values: 44-80 µmol/l

3. Results and discussion

The mean results ± SD of creatinine determination are shown in Table. 1-2 and illustrated in Fig. 1-2. Investigations revealed that the drugs raised plasma creatinine levels in a concentration dependent manner which was not time dependent, with Microgynon having the highest increase of 300% for the highest dose of 3.6 µg/100g body wt. (354.00 ± 0.00 vs control 88.50 ± 0.00 µmol/l) followed by Primolut (236.00 ± 29.50 vs control 88.50 ± 0.00 µmol/l). The differences in dosage were statistically significant on the effect of the drug on the plasma creatinine levels at 95.0% confidence level (P< 0.05). A report by Smith and Sizto (1983) suggests that high progestogen increases serum ceatinine. Creatinine is a protein breakdown product. Its level is a reflection of the bodies muscle mass. Low levels are commonly seen in inadequate protein intake, liver disease, kidney damage or pregnancy.
Table 1. _Invivo effect of Microgynon on rat plasma creatinine expressed in μmol/l_

<table>
<thead>
<tr>
<th>Microgynon</th>
<th>Creatinine (μmol/l)</th>
<th>2hrs</th>
<th>4hrs</th>
<th>24hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 (Control)</td>
<td>88.5 ± 0.00</td>
<td>88.5 ± 29.50</td>
<td>88.5 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>0.36</td>
<td>118 ± 29.50</td>
<td>118 ± 0.00</td>
<td>118 ± 29.50</td>
<td></td>
</tr>
<tr>
<td>0.72</td>
<td>177 ± 29.50*</td>
<td>117 ± 0.00*</td>
<td>117 ± 29.50*</td>
<td></td>
</tr>
<tr>
<td>1.40</td>
<td>236 ± 0.00*</td>
<td>236 ± 29.50*</td>
<td>250 ± 0.00*</td>
<td></td>
</tr>
<tr>
<td>1.80</td>
<td>265 ± 29.50*</td>
<td>265 ± 0.00*</td>
<td>265 ± 29.50*</td>
<td></td>
</tr>
<tr>
<td>3.60</td>
<td>354 ± 0.00*</td>
<td>354 ± 29.50*</td>
<td>354 ± 0.00*</td>
<td></td>
</tr>
</tbody>
</table>

Results are means of three determinations ± standard deviation.* Statistically significant at 95.0% confidence level. (P< 0.05)

Table 2. _Invivo effect of Primolut N on rat serum creatinine expressed in μmol/l_

<table>
<thead>
<tr>
<th>Primoluta</th>
<th>Creatinine (μmol/l)</th>
<th>2hrs</th>
<th>4hrs</th>
<th>24hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 (control)</td>
<td>85.55 ± 0.00</td>
<td>88.5 ± 0.00</td>
<td>88.5 ± 29.50</td>
<td></td>
</tr>
<tr>
<td>10.00</td>
<td>88.5 ± 0.00</td>
<td>88.5 ± 0.00</td>
<td>88.5 ± 29.50</td>
<td></td>
</tr>
<tr>
<td>20.00</td>
<td>153.4 ± 2.95*</td>
<td>150.45 ± 2.95*</td>
<td>147.5 ± 29.50*</td>
<td></td>
</tr>
<tr>
<td>40.00</td>
<td>179.95 ± 2.95*</td>
<td>179.95 ± 2.95*</td>
<td>177 ± 0.00*</td>
<td></td>
</tr>
<tr>
<td>50.00</td>
<td>212.4 ± 2.95*</td>
<td>206.5 ± 29.50*</td>
<td>206 ± 29.50*</td>
<td></td>
</tr>
<tr>
<td>100.00</td>
<td>241.9 ± 2.95*</td>
<td>238.95 ± 0.00*</td>
<td>236 ± 29.50*</td>
<td></td>
</tr>
</tbody>
</table>

Results are means of three determinations ± standard deviation. * Statistically significant at 95.0% confidence level. (P< 0.05)

Elevated levels are generally reflective of kidney damage and need to be monitored very carefully. Creatinine is derived from creatine and creatine phosphate in a muscle tissue and may be defined as a nitrogenous waste product. Creatinine is not reutilized but is excreted from the body in the urine via the kidney. It is produced and excreted at a constant rate which is proportional to the body muscle mass. Because of the way in which creatinine is excreted by the kidney, creatinine measurement is used almost exclusively in the assessment of kidney function. Creatinine is regarded as the most useful endogenous marker in the diagnosis and treatment of kidney disease.

Creatinine is measured primarily to assess kidney function and has certain advantages over the measurement of urea. The plasma level of creatine is relatively independent of protein ingestion, water intake, rate of urine production and exercise. Since its rate of production is constant, elevation of plasma creatinine is indicative of under excretion, suggesting kidney impairment. Depressed levels of plasma creatinine are rare and not clinically significant (Briggs, 1976; Grinspoon SK et al., 2003). From these results, it is advisable that women go for kidney function tests in the laboratory, before taking these oral contraceptives. They should also go undergo these tests every six months while taking the oral contraceptives and after they have stopped taking the oral contraceptives.

4. Conclusion

Intake of Microgynon and Primolut-N increased the rat plasma creatinine levels with a number of possible implications by extrapolation on the female ‘homo sapiens’. Full medical laboratory tests should be undergone before prescription of these drugs. The tests must include kidney function. There should be check up tests every six months.

5. Acknowledgement
The authors are grateful to the Animal House Unit of the Department of Biochemistry, University of Port Harcourt, Nigeria for the provision of the experimental animals used for this work.

6. References


