Effect of Microgynon, Primolut-N and Postinor on plasma total protein of Wistar Albino Rat (*Rattus rattus)*

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Abstract

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 *Invivo* effects on wistar albino rat, plasma total protein. Plasma total protein is a measure of the total amount of proteins in the blood. Studies showed that plasma total protein was decreased by the drugs with Microgynon having the highest decrease (0.57 ± 0.19 g/l) ($P < 0.05$) followed by Primulot (0.95 ± 0.19 g/l) and then Postinor (1.14 ± 0.19 g/l). The random use of the drugs in our society today especially as most women abuse these drugs demands for more biochemical research to elucidate the effects of these drugs not only on the hormones but also, on other biochemical parameters like the plasma total protein. The obtained results indicate that women should use these drugs only after physicians advice.

Keywords: Microgynon, Primolut- N, Total protein

1. Introduction

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 for contraception and also for other non contraceptive benefits. The drugs are now used by 50 million to 100 million women throughout the world. (kay *et al.,* 1974; Kuhl & Goethe, 1990; CHPE, 1984). The National Cancer Institute (2003) reported that there are currently, two types of OCs available. The most commonly prescribed OC contain two commercially available female hormones (estrogen & progesterone) that are similar to the hormones secreted by ovaries.

Estrogen stimulates the growth and development of the uterus during puberty, causes the endometrium (the inner lining of the uterus) to thicken during the first half of the menstrual cycle, and influences breast tissue throughout life, but particularly from puberty to menopause. Progesterone, which is produced during the last half of the menstrual cycle, prepares the endometrium to receive the egg. If the egg is fertilized, progesterone secretion continues, preventing release of additional eggs from the ovaries. For this reason, progesterone is called the "pregnancy supporting" hormone and scientists believe that it has valuable contraceptive effects. Commercially available progesterone used in OCs is called progesterone or progestin.

The combined formulations are the most widely used and most effective. The combination phasic formulations contain two or three different amounts of the same estrogen and progestin. Each of the tablets containing one of these various dosages is given for intervals varying from 5 - 11 days during the 21st day medication period. These formulations have been described as biphasic or triphasic and are generally referred to as multiphasic. The rationale for this type of formulation is that a lower total dose of steroid is administered without increasing the incidence of breakthrough bleeding. In the usual regimen for combination OCs, no medication is given for 1 week out of 4 to allow withdrawal bleeding to occur.

The second type of OC available is called the mini-pill. It contains only a progestogen. The mini-pill is less effective in preventing pregnancy than the combination pill . However, if the mini-pill is taken absolutely regularly each day within a time span of a couple of hours, without breaks and regardless of bleeding patterns, they are almost as effective as combined oral contraceptive, especially for those aged 30 and over. Like any other drugs, they have some side effects ranging from nausea to cancer. Initially, oral contraceptive formulations contained very high levels of synthetic estrogen and progesterone, based on the assumptions that these levels were necessary to prevent pregnancy (Skouby & Jesperson, 1990). Over the years however, hormone levels have continually decreased in order to provide formulation with maximum efficiency and minimum side ef-

fects (Grimes *et al.,* 1993).

Plasma total protein is a measure of the total amount of protein in the blood. The random use of the drugs in our society today especially as most women abuse these drugs demands for more biochemical research to elucidate the effects of these drugs not only on the hormones but also on other biochemical parameters like the plasma total protein. This is therefore the focus of the present study.

2. 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. Postinor was bought from Chemical works of Gedeon Richter Ltd. Budapest Hungary. Reagent kit for the total protein was from Human Gesellschaft fur Biochemica und Diagnostica mbH, Max-Planck-Ring 21- D-65205 Wiesbaden - Germany.

162 albino rats (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 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 55 kg. 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 and 15.00µg 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 hrs intervals ranging from 2 hrs, 4 hrs and 24 hrs. 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 collections were done immediately and were stored in a lithium heparin sample containers. The blood was centrifuged at 3000 rotations per minute for 3 mins and the blood plasma were separated and used for the assay.

2.1 Blood total protein

Total protein levels were determined by Buiret method (colorimetric test). The Principle of this method is that cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample (Weichselbaum, 1946).

Reagent kit contained Sodium hydroxide (200mmol/l), Potassium sodium tartrate (32 mmol/l), Copper sulphate (12 mmol/l), Potassium iodide (30mmol/l). Standard contained Protein (80g/l), Sodium azide (0.095%). Sodium tetraphenyl boron (2.1 mmol/l), Potassium standard (4 mEq/L).

One millilitre of reagent was mixed with 0.02 ml of the sample. The standard tube contained 1.00 ml of reagent and 0.02 ml of the standard. The mixtures were incubated for 10 mins. The absorbance of the sample and standard were read against the reagent blank within 30 mins at 546nm with spectronic -20 spectrophotometer.

2.2 Calculations

$$\text{Protein concentration (C)} = 80 \times \frac{\Delta A_{sample}[g/l]}{\Delta A_{STD}}$$

3. Results and discussion

The mean test results ± SD of plasma total protein determinations are shown on tables 1 to 3 (fig. 1- fig. 3). The drugs had a decreasing effect on the blood total protein. Microgynon had the highest decrease (0.57 ± 0.19 vs control 1.90 ± 0.00 g/l) followed by Primolut (0.95 ± 0.19 vs control 1.90 ± 0.00 g/l) and then Postinor (1.14 ± 0.19 *Vs* 1.90 ± 0.19 g/l). Plasma total protein is a measure of the total amount of protein in the blood. A low or high total protein does not indicate a specific disease, but it does indicate that some additional tests may be required to determine if there is a problem (Briggs, 1976).

The synthetic estrogens used in OCs cause an increase in the hepatic production of several globulins, some of which are involved in the coagulation process. Secondly, angiotensinogen may be converted to angiotensin and increase blood pressure in some users (Wilson *et al.*, 1984). The circulating levels of each of these globulins are directly correlated with the

amount of estrogen in the OC formulation. Epidemiologic studies have shown that the incidence of both venous and arterial thombosis is also directly related to the dose of estrogen (Bottigger *et al.*, 1980; Meade *et al.*, 1980; Mann *et al.*, 1986).

Fig. 1 . *Effect of Microgynon on total protein*

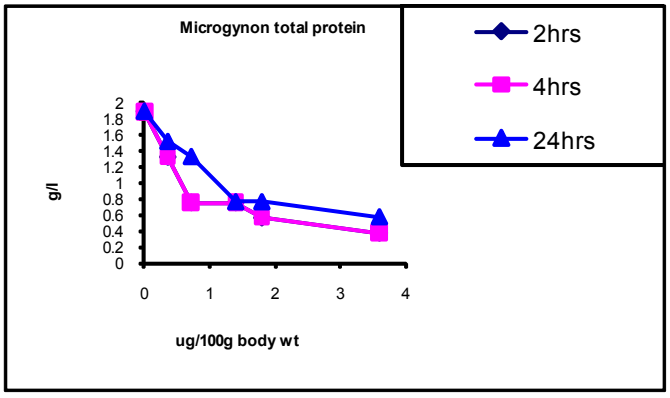

Fig. 2 . *Effect of Primolut- N on total protein*

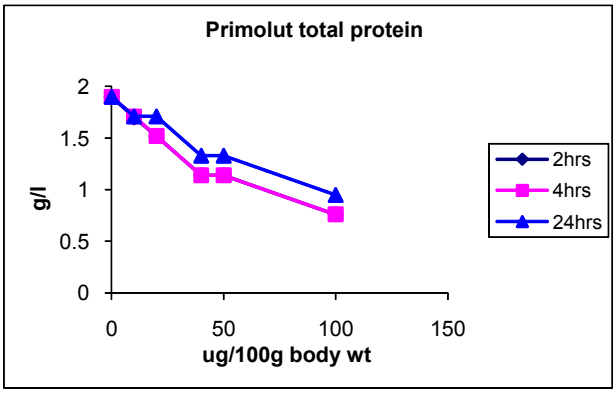

Fig. 3 *Effect of Postinor on total protein*

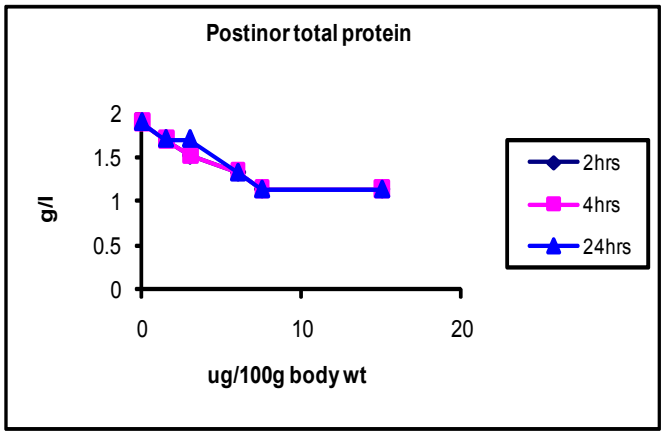

Table. 1 I*nvivo effect of Microgynon on rat plasma total protein expressed in g/l*

Microgyno-na µg/100g body wt	Plasma Total protein (g/l)		
	2hrs	4hrs	24hrs
0.00	1.9 ± 1.16	1.9 ± 0.00	1.9 ± 0.00
0.36	1.33 ± 0.19	1.33 ± 0.38	1.52 ± 0.00
0.72	0.76 ± 0.00	0.76 ± 0.00	1.33 ± 0.19
1.40	0.76 ± 0.38	0.76 ± 0.38	0.76 ± 0.19
1.80	0.57 ± 0.19	0.57 ± 0.19	0.76 ± 0.19
3.60	0.38 ± 0.19	0.38 ± 0.19	0.57 ± 0.19
Results are means of three determinations ± standard deviation			

Table 2. *In vivo effect of Primolut -N on rat plasma total protein expressed in g/l*

Primolut-N µg/100g	Plasma Total protein (g/l)		
body wt	2hrs	4hrs	24hrs
0.00	1.9 ± 0.00	1.9 ± 0.00	1.9 ± 0.00
10.00	1.7 ± 0.00	1.71 ± 0.00	1.71 ± 0.00
20.00	1.52 ± 0.19	1.52 ± 0.19	1.71 ± 0.19
40.00	1.14 ± 0.19	1.14 ± 0.00	1.33 ± 0.19
50.00	1.14 ± 0.00	1.14 ± 0.19	1.33 ± 0.19
100.00	0.76 ± 0.19	0.76 ± 0.19	0.95 ± 0.19
Results are means of three determinations ± standard deviation			

Table 3. *In vivo effect of Postinor on rat plasma total protein expressed in g/l*

Postinor µg/100g body wt	Plasma Total protein (g/l)		
	2hrs	4hrs	24hrs
0.00	1.9 ± 0.00	1.9 ± 0.00	1.9 ± 0.19
1.50	1.71 ± 0.19	1.71 ± 0.19	1.71 ± 0.00
3.00	1.52 ± 0.19	1.52 ± 0.19	1.71 ± 0.19
6.00	1.33 ± 0.19	1.33 ± 0.19	1.33 ± 0.19
7.50	1.33 ± 0.19	1.14 ± 0.19	1.14 ± 0.19
15.00	1.14 ± 0.19	1.14 ± 0.19	1.14 ± 0.19
Results are means of three determinations ± standard deviation			

4. Conclusion

From this study, it was found out that these oral contraceptives studied, tend to decrease the total blood proteins. It is recommended that full medical laboratory tests should be undergone before prescription of these drugs. We suggest the following parameters such as liver function, kidney function and full blood analysis as preventive measures. Individuals who are using these drugs should undergone check up tests every six months once. Blood pressure and body weight should be measured, and a physical examination (including breast, abdominal, and pelvic examination with cervical cytology) performed.

5. Acknowledgement

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6. References

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