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Invivo effect of microgynon and primolut-N on plasma sodium and potassium of wistar albino rat.

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Abstract

Microgynon (0.15mg levonorgestrel and 0.03mg ethinylestradiol), is an oral contraceptives Primolut -N a mini pill (5mg norethisterone) were analysed for their in-vivo effects on albino rat plasma sodium and potassium levels. Studies showed that the drugs decreased sodium levels with microgynon showing the highest decrease of 29.85% at highest concentration level of 3.6µg /100g (94.00 ± 28.50mmol/l) ($P < 0.05$), followed by Primulot-N (102 ± 28.50 mmol/l). Investigations also revealed that the drugs decreased plasma potassium levels. Microgynon had the highest decrease of 88.88% for the highest dose of 3.60µg/100g body wt, while the lowest decrease of 22.22% was observed for the lowest dose of 0.36µg/100g).The sodium element plays an important role in salt and water balance in the body. The metabolism of sodium and potassium is closely linked with the maintenance of fluid balance and with the regulation of acid-base status, Elevated levels are related to acidosis as well as too much water crossing the cell membrane. The potassium element is found primarily inside the cells of the body. 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 body electrolytes. This result indicates that laboratory tests are needed for women before using these drugs.

Key Words: Microgynon, Primulot-N, Plasma Sodium and Potassium

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. 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 drugs, 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 & Jesperson, 1990). 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. Finally, they may alter ovarian responsiveness to gonadotropin stimulation. Nevertheless, neither gonadotropin production nor ovarian steroidogenesis is completely

abolished. Levels of endogenous estradiol in the peripheral blood during ingestion of combination OCs are similar to those found in the early follicular phase of the normal cycle (Mishell *et al*., 1982). Contraceptive steroids prevent ovulation mainly by interfering with release of gonadotropin-releasingHormne (GnRH) from the hypothalamus.

The combination OCs probably does 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. The effect is more pronounced with formulations containing a more potent progestin and with those containing 50 μg or more of estrogen than with 30- to 35 μg formulations (Scott *et al*., 1978). There are data showing that the delay in the resumption of ovulation after discontinuation of OC use is shorter in women ingesting preparations with less than 50 μg of estrogen than I those ingesting formulations with 50 μg of estrogen or more (Bracken *et al*., 1990).World Health Organization (1982) reported that the daily progestin-only preparations do not consistently inhibit ovulation, because of the inconsistent ovulation inhibition, their effectiveness is significantly less than that of the combination type of OCs.

The sodium element plays an important role in salt and water balance in the body. The metabolism of sodium and potassium is closely linked with the maintenance of fluid balance and with the regulation of acid-base status (Wootton & Freeman, 1982). Elevated levels are related to acidosis as well as too much water crossing the cell membrane. The potassium element is found primarily inside the cells of the body. 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 body electrolytes. This is therefore the focus of the present study.

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. The sodium reagent kit was bought from Quimica Clinica Aplicada S.A. A7 Km 1081 – P.O Box 20 – E43870 Amposta/ Spain. While the potassium reagent kit was bought from Linear Chemicals S.L (Cromatest) Joaquim Costa, 18, 2a planta. 08390 Montgat-Barcelona, Spain.

108 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 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 and 100.00 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 2 hours, 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 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.

Sodium levels were determined by colorimetric test. Magnesium-uranyl acetate method. The Principle of this method is that after the precipitation of sodium magnesium-uranyl acetate, in the supernatant form with uranyl ions in solution with thioglycolic acid a yellow-brown coloured complex is formed. The optical density difference between the reagent blank (without precipitation of sodium) and the result of the analysis is proportional to the sodium concentration (Trinder, 1951).

Reagent A kit contained uranylacetate (19mM) and magnesium acetate (140mM) while reagent B kit contained ammonium thioglycolate (550mM), ammonia (550mM) and the standard aqeous solution of sodium equivalent150mmol.

2.00ml of reagent A was mixed with 0.02 ml of the sample. For the standard, 2.00 ml of reagent A and 0.02 ml of the standard were mixed. The mixtures were let to stand for 5 minutes, they were then shaken thoroughly for 30 seconds. The mixtures were allowed to stand for 30 minutes. They were centrifuged at 2,000rpm for 5 minutes. The supernatant was then separated. 0.05ml of the clear supernatant was mixed with 2.00ml of reagent B. For the blank, 0.05 ml of reagent A and 2.00 ml of reagent B were mixed, while the standard tube contained 0.05 ml of supernatant and 2.00ml of reagent B. The absorbance of the mixtures was read after 10 minutes at 405nm with spectronic – 20 spectrophotometer.

Calculations

$$\frac{\text{Blank O.D - Sample O.D}}{\text{Blank O.D - Standard O.D}} \times 150 = \text{mmol sodium/L}$$

Normal values 135-150 mmol/l.

Potassium levels were determined by colorimetric endpoint method. The Principle of this method is that the amount of potassium is determined by using sodium tetraphenylboron (2.1mmol/l) in a specifically prepared mixture to produce a potassium concentration in the range of 2 – 7 mEq/L (Terri & Sesin, 1958). 1.0ml of reagent was mixed with 0.1ml of sample except for the controls, which had no samples. The blank tube contained 1.0ml of reagent while the standard tube contained 1.0ml of ragent and 0.1ml of standard. The mixtures were incubated at 25^{o}C for 3mins.
The absorbance was read against reagent blank at 500nm with Spectronic -20 spectrophotometer.

Calculations

$$\frac{\Delta A_{unknown}}{\Delta A_{standard}} \times C_{standard} = \text{Potassium concentration (mEq/L)}$$

Results and discussion

The mean results ± SD of sodium determinations are shown on tables 1 and 2 (figures 1 and 2). While that of potassium determinations are shown on tables 3 and 4 (figures 3 and 4). It was observed that the drugs decreased plasma sodium levels in a concentration dependent manner. The effect of the drugs on plasma sodium levels did not depend on time as the difference in the readings was not significant. Studies on plasma sodium level showed that the drugs decreased sodium levels with Microgynon showed the highest decrease (94.00 ± 28.50 vs. control 134.00 ± 22.25 mmol/l) then Primolut (102.00 ± 28.50 vs. control 133.00 ± 27.90 mol/l). The sodium element plays an important role in salt and water balance in the body. A low level in the blood can be caused by too much water intake, heart failure, or kidney failure. A low level can also be caused by loss of sodium in diarrhea fluid, or vomiting. A high level can be caused by too much intake of salt or by not enough intake of water (Ghoneim, *et al.*, 1975).

Investigations also revealed that the drugs decreased plasma potassium levels in a concentration and time dependent manner. The highest decrease was observed at 2hours intervals while the lowest decrese was observed at 24 hrs. Microgynon and Primolut showed the highest decrease (0.05 ± 0.00 vs. control 0.46 ± 0.00 mEq/l) Smith and Sizto (1983) revealed that high progestogen increases serum sodium and potassium levels in women. Henderson et al. (1991) stipulated that the levels of hormones in oral contraceptives have decreased steadily over

the years. This might explain why the drugs did not increase the sodium and potassium levels in rat, they had a decreasing effect. This is good for the sodium aspect but not good for the potassium component. The potassium element is found primarily inside the cells of the body. Low levels in the blood may indicate severe diarrhea, alcoholism, or excessive use of water pills. Low potassium levels can cause muscle weakness and heart problems. Potassium is the principle cation of the intracellular fluid.

It is also an important constituent of the extracellular fluid due to its influence on muscle activity. Its intracellular function parallels that of its extracellular function, namely influencing acid-base balance and osmotic pressure, including water retention (Henry, R.F. 1974; Tietz, N. W. 1958). Elevated potassium levels (hyperkalemia) are often associated with renal failure, dehrdration, shock or adrenalin insufficiency. Decreased potassium levels (hypokalemia) are associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex (Ghoneim *et al.*, 1975). From the result s obtained from this study, women who are hypertensive should not take the drugs and non-hypertensive women placed on the drugs should have their blood pressure monitored from time to time, possibly every three months to ascertain that their potassium levels do not go too low during the course of their taking the drugs (Fig 1-4).

Conclusion

From this study, it was observed that the intake of Microgynon and Primolut-N decreased plasma
sodium and potassium levels. It is recommended that full medical laboratory tests be undergone before prescription of these drugs. The tests should include liver function, kidney function and full blood analysis. There should be check up tests every six months.

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Fig.1.Illustrates the effect of Microgynon on Na^+ (mmol/l)

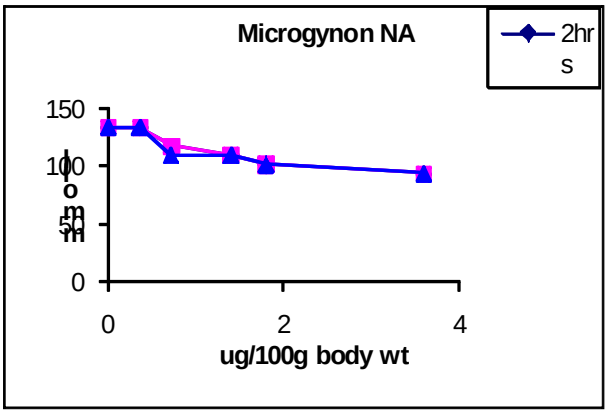

Fig.2. illustrates the effect of Primolut - N on Na^+ (mmol/l)

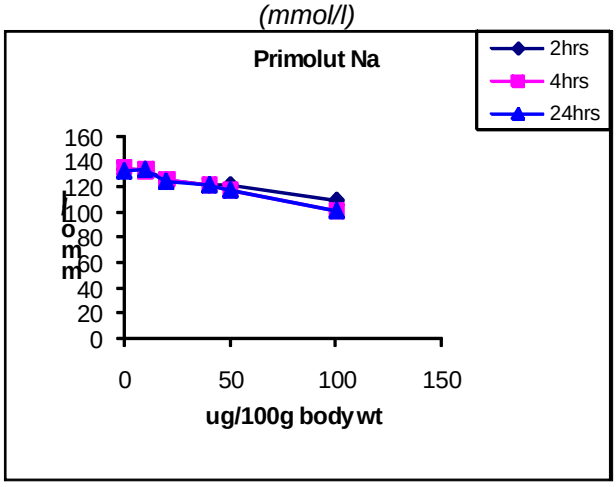

Fig.3. illustrates the effect of Microgynon on K^+ (mEq/l)

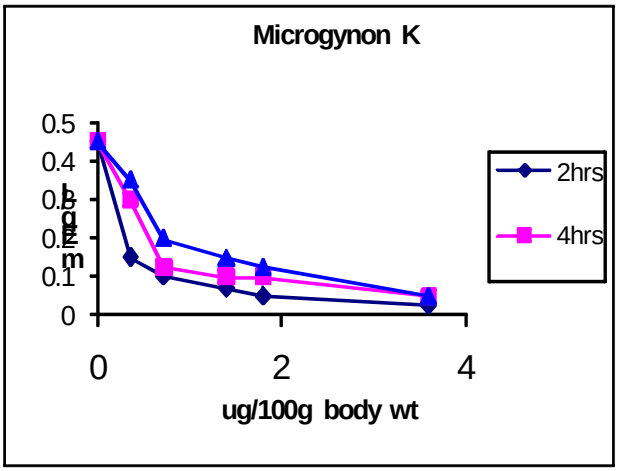

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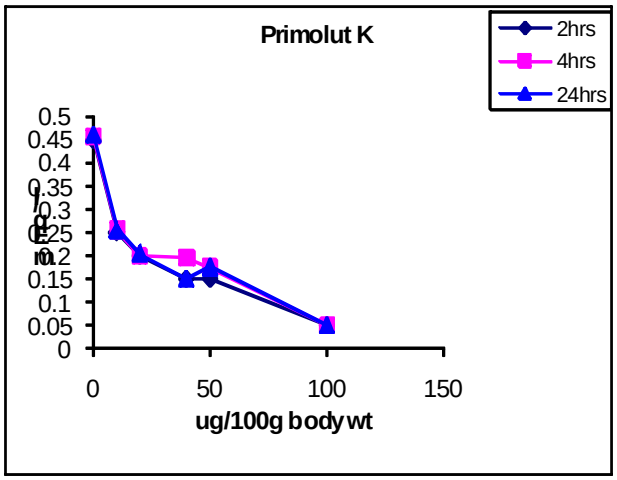
Fig.4.Effect of Primolut on K^+ (mEq/l)



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