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A study of the *invivo* effect of microgynon and primolut-N on albino rat plasma aspartate amino transferase (EC 2.6.1.1) and alanine amino transferase (EC 2.6.1.2) AT 37 ^{O}C, pH = 9.8.

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Abstract

Oral contraceptives namely Microgynon a combined pill (0.15mg levonorgestrel and 0.03mg ethinylestradiol) and Primolut -N a mini pill (5mg norethisterone) were analysed for their in-vivo effects on albino rat plasma and erythrocyte aspartate amino transferase (AST). The in-vivo effects of the oral contraceptives on albino rat plasma and erythrocyte AST showed that the drugs inhibited the activity of the enzymes in a concentration dependent manner. The effect of the drugs on the enzymes were also time dependent with the highest inhibition obtained at 24 hours duration while the least inhibition occurred at 2 hours duration Microgynon showed the highest inhibition (7.00 ± 0.00 vs. control 31.00 ± 0.00 U/L) ($P < 0.05$) followed by Primolut (16.00 ± 0.00 vs. control 27.00 ± 4.00 U/L). The erythrocyte AST activity was also inhibited. The highest inhibition values obtained were Microgynon (36.00 ± 0.00 U/L) then Primolut (41.00 ± 0.00 U/L). The least inhibition values obtained were Microgynon and Primolut (67.00 ± 0.00). The in-vivo effects of the oral contraceptives on rat plasma and erythrocyte ALT showed that the drugs activated the activity of the enzymes in a concentration dependent manner. The effect of the drugs on the enzymes were also time dependent with the highest activation obtained at 24 hours duration while the least activation occurred at 2 hours duration. Primolut showed the highest activation (18.00 ± 0.00 U/L). The erythrocyte enzymes showed higher activity than the plasma enzymes. Microgynon showed the highest activity (50.00 ± 2.00 U/L). This result indicates that liver function tests are needed for women before using these drugs.

Key Words: Microgynon, Primolut –N

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. 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 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 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).

Transamination is the term given to the process in which an amino group is transferred from α -amino acid to α-Keto acid. As a result, a different α -amino acid and a different α-Keto acid are formed. All naturally occurring α – amino acid can take part in such reaction where different enzymes are involved. Enzymes generally known as amino transferase or transaminase catalyse in reactions. Most require α-Keto glutarate as one amino acid group acceptor; they are therefore specific for the substrate couple α-

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Ketoglutarate-L-glutamate. The most prominent of all the transamination reaction is that which causes the amination for the-Ketoglutarate to glutamic acid.

Aspartate transaminase (SGOT) is the enzyme that catalyses this reaction. Aspartate transaminase (SGOT) like other transaminses has its prosthetic group, pyridoxal phosphate. This prosthetic group links with the enzyme non-covalently through a charged nitrogen atom. Generally, glutamic acid acts as an intermediary for the disposal of α-amino nitrogen of most of the amino acid in transamination. Alanine transaminase is widely distributed in most mammalian tissues such as the liver, heart, skeletal muscles, brain, and kidney, but its concentration in tissue other than the liver is relatively low.

This enzyme, together with other transaminase enzymes example aspartate trasaminase and some is enzymes are particularly useful in medical diagnosis, which involves the identification of diseases from the examination of symptom in relation to their presence in the serum erythrocytes, leucocytes and other body fluids. These enzymes have become increasingly important in clarifying the etiology, pathonogenesis as well as diagnosis of a number of diseases associated with the different organ in which they are present or concentrated.

However, the presence of alanine transaminase and aspartate transaminase in the serum and to a lesser extent in the other body fluids is as a result of their releases from the site of production. Abnormal values of serum alanine transaminase have been observed for different disease conditions such as liver function or hepatic diseases. The apparent or presumed mechanisms responsible for such observation include an increase in its rate of release from tissue, an increase in the amount available for release, or decrease in its rate of disposition.

Materials and method

Microgynon was bought from Schering AG Germany; Primolut- N was bought from Medipharm (Pvt) Ltd., Lahore, Licensee of Schering AG. Federal Republic of Germany, Reagent kits were bought from Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom BT29 4QY.

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, 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 was 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 assay. While the remaining erythrocyte was used for the determination of the erythrocyte enzymes.

Aspartate reagent kit contained 1. Buffer: Phosphate buffer (100mmol/l, pH 7.4), L-aspartate (100mmol/l), α- oxoglutarate (2 mmol/l). 2.: 2, 4-dinitrophenylhydrazine (2mmol/l).

Aspartate levels were determined by colorimetric test. The Principle of this method is that Glutamic-Oxaloacetic Transaminase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine (Reitman & Frankel, 1957).

Solution 1 (0.5ml) was mixed with 0.1 ml of the sample. The blank tube contained 0.5 ml of solution 1 and 0.1 ml of distilled water. The mixtures were incubated for 30 mins at 37^{o}C. 0.5ml of solution 2 was then added to the tubes. They were then allowed to stand for 20 minutes at 25^{o}C. 5.00ml of sodium hydroxide was added to the tubes. The absorbance of the mixture was read against the reagent blank after 5mins using a 1cm light path cuvette at 546nm with spectronic-20 spectrophotometer.

Aspartate levels were determined by colorimetric test. The Principle of this method is that Glutamic-Oxaloacetic Transaminase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine (Reitman & Frankel, 1957). Alanine reagent kit contained, 1. Buffer: Phosphate buffer (100mmol/l, pH 7.4), L-alanine (100mmol/l), α- oxoglutarate (2mmol/l). 2. 2,4-dinitrophenylhydrazine (2mmol/l).

Alanine levels were determined by colorimetric test. The Principle of this method is that Glutamic-Pyruvic Transaminase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine (Reitman & Frankel,1957).

Solution 1 (0.5ml) was mixed with 0.1 ml of the sample. The blank tube contained 0.5 ml of solution 1 and 0.1 ml of distilled water. The mixtures were incubating for 30 minutes at 37^{o}C. 0.5ml of solution 2 was then added to the tubes. They were then allowed to stand for 20 mins at 25^{o}C. 5.00ml of sodium hydroxide was added to the tubes. The absorbance of the mixture was read against the reagent blank after 5 minutes using a 1cm light path cuvette at 546nm with spectronic-20 spectrophotometer.

Results and discussion

Mean results ± SD of the in vivo effects of the oral contraceptive drugs on plasma and erythrocyte Aspartate aminotranserase and Alanine aminotransferase are shown in Tables 1-8. These enzymes are primarily found in the liver. The results showed that the drugs in comparison with the controls inhibited the activity of rat plasma AST. The drugs inhibited the activity of the enzyme in concentration dependent manner. The effect of the drugs on the enzymes were also time dependent with the highest inhibition obtained at 24 hours duration while the least inhibition occurred at 2 hours duration. Microgynon showed the highest inhibition (7.00 ± 0.00 vs. control 31.00 ± 0.00 IU/L) followed by Primolut -N (16.00 ± 0.00 vs. control 27.00 ± 4.00 IU/L).

Table 1.Invivo effect of Microgynon on rat plasma Aspartate Aminotransferase activity (EC 2.6.1.1) at 37^{o}C, pH = 9.8. expressed in IU/L

Microgynona µg/100g b /wt	Plasma AST Activity (IU/L)		
	2hrs	4hrs	24hrs
0.00	31 ± 4.00	31 ± 4.00	31 ± 0.00
0.36	27 ± 4.00	23 ± 4.00	19 ±4.00
0.72	19 ± 0.00	16 ± 4.00	16 ± 7.00
1.40	16 ± 2.00	14 ± 0.00	14 ± 3.00
1.80	16 ± 0.00	13 ± 2.00	13 ± 2.00
3.60	10 ± 4.00	7 ± 4.00	7 ± 0.00
Results are means of three determinations ± standard deviation			

Table 2.Invivo effect of Microgynon on rat erythrocyte Aspartate Aminotransferase activity (EC 2.6.1.1) at 37^{o}C, pH = 9.8 expressed in IU/L

Microgynon µg/100g b.wt	Erythrocyte AST Activity (IU/L)		
	2hrs	4hrs	24hrs
0.00	67 ± 4.00	67 ± 4.00	67 ± 0.00
0.36	67 ± 4.00	67 ± 0.00	59 ± 4.00
0.72	59 ± 7.00	59 ± 4.00	52 ± 4.00
1.40	52 ± 4.00	52 ± 0.00	47 ± 7.00
1.80	47 ± 7.00	47 ± 0.00	41 ± 7.00
3.60	41 ± 4.00	36 ± 4.00	36 ± 0.00
Results are means of three determinations ± standard deviation			

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Table 3. In vivo effect of Primolut -N on rat plasma Aspartate Aminotransferase activity (EC 2.6.1.1) at 37°C , pH = 9.8 expressed in IU/L

Primolut - N µg/100g b/ wt	Plasma AST Activity (IU/L)		
	2hrs	4hrs	24hrs
0.00	31 ± 4.00	31 ± 4.00	27 ± 4.00
10.00	29 ± 0.00	29 ± 0.00	27 ± 4.00
20.00	25 ± 0.00	23 ± 4.00	23 ± 7.00
40.00	23 ± 4.00	23 ± 4.00	21 ± 0.00
50.00	19 ± 4.00	19 ± 7.00	19 ± 0.00
100.00	16 ± 4.00	16 ± 0.00	16 ± 0.00

Table 4. Invivo effect of Primolut -N on rat erythrocyte Aspartate Aminotransferase activity (EC 2.6.1.1) at 37°C , ph = 9.8 expressed in IU/L.

Primolut -N	Erythrocyte AST Activity (IU/L)		
µg/100g b.wt	2hrs	4hrs	24hrs
0.00	76 ± 7.00	76 ± 0.00	67 ± 7.00
10.00	67 ± 0.00	59 ± 0.00	59 ± 7.00
20.00	59 ± 4.00	52 ± 4.00	52 ± 0.00
40.00	59 ± 4.00	59 ± 7.00	52 ± 7.00
50.00	47 ± 0.00	41 ± 0.00	47 ± 4.00
100.00	41 ± 4.00	41 ± 4.00	41 ± 0.00

Results are means of three determinations ± standard deviation

Table 5. In vivo effect of Microgynon on rat plasma Alanine Aminotransferase activity (EC 2.6.1.2) at 37°C , pH = 9.8 expressed in IU/L

Microgynona	Plasma ALT Activity (IU/L)		
µg/100g b.wt	2hrs	4hrs	24hrs
0.00	6 ± 1.00	6 ± 0.00	6 ± 2.00
0.36	10 ± 1.00	13 ± 2.00	16 ± 2.00
0.72	13 ± 1.00	13 ± 0.00	16 ± 2.00
1.40	16 ± 3.00	17 ± 0.00	16 ± 1.00
1.80	17 ± 0.00	16 ± 0.00	17 ± 0.00
3.60	17 ± 0.00	17 ± 0.00	16 ± 0.00

Table 6.In vivo effect of Microgynon on rat erythrocyte Alanine Aminotransferase (EC 2.6.1.1) at 37°C, pH = 9.8 expressed in IU/L

Microgynon	Erythrocyte ALT Activity (IU/L)		
µg/100g b.wt	2hrs	4hrs	24hrs
0.00	27 ± 2.00	27 ± 0.00	27 ± 2.00
0.36	28 ± 2.00	27 ± 3.00	28 ± 3.00
0.72	34 ± 0.00	34 ± 2.00	35 ± 2.00
1.40	36 ± 3.00	36 ± 0.00	40 ± 2.00
1.80	43 ± 2.00	43 ± 2.00	45 ± 2.00
3.60	49 ± 2.00	49 ± 0.00	50 ± 2.00

Table 7. In vivo effect of Primolut -N on rat plasma Alanine Aminotransferase (EC 2.6.1.2) at 37°C, pH = 9.8 expressed in IU/L

Primulot-N	Plasma ALT Activity (IU/L)		
µg/100g b.wt	2hrs	4hrs	24hrs
0.00	5 ± 0.05	6 ± 2.00	6 ± 0.00
10.00	8 ± 2.00	9 ± 2.00	9 ± 0.00
20.00	9 ± 2.00	10 ± 2.00	13 ± 2.00
40.00	10 ± 0.00	11 ± 0.05	14 ± 0.05
50.00	13 ± 0.00	14 ± 2.00	17 ± 0.00
100.00	14 ± 0.00	17 ± 0.00	18 ± 0.00

Table 8. Invivo effect of Primolut -N on rat erythrocyte Alanine Aminotransferase (EC 2.6.1.2) at 37°C , pH = 9.8 expressed in IU/L

Primolut -N	Erythrocyte ALT Activity (IU/L)		
µg/100g b.wt	2hrs	4hrs	24hrs
0.00	27 ± 2.00	27 ± 2.00	28 ± 0.00
10.00	30 ± 2.00	30 ± 2.00	32 ± 2.00
20.00	32 ± 0.00	32 ± 2.00	34 ± 0.00
40.00	34 ± 2.00	35 ± 0.00	36 ± 2.00
50.00	35 ± 2.00	36 ± 2.00	40 ± 2.00
100.00	40 ± 2.00	38 ± 2.00	43 ± 2.00

The erythrocyte AST activity was also inhibited. The highest inhibition values obtained were Microgynon (36.00 ± 0.00 IU/L) then Primolut -N (41.00 ± 0.00 IU/L). The least inhibition values obtained were Microgynon and Primolut -N (67.00 ± 0.00 IU/L). (Ohno *et al*., 1994) reported no increase in AST activity in rats fed DT 5061 an oral contraceptive. The *invivo* effects of the oral contraceptives on rat plasma and erythrocyte ALT showed that the drugs activated the activity of the enzymes in a concentration and time dependent manner.

The effect of the drugs on the enzymes were also time dependent with the highest activation obtained at 24 hours duration while the least activation occurred at 2 hours duration. Primolut -N showed the highest activation (18.00 ± 0.00 vs. control 6.00 ± 0.00 IU/L) followed by Microgynon (16.00 ± 0.00 vs. control 6.00 ± 2.00 IU/L). The erythrocyte enzymes showed higher activity than the plasma enzymes. The highest activity was shown by Microgynon (50 ± 2.00 vs. control 27 ± 2.00 IU/L) then Primolut -N (43 ± 2.00 vs. control 28 ± 0.00 IU/L).

Drinking too much alcohol, certain drugs, liver disease and bile duct disease can cause high levels in the blood. Hargreaves (1969) that oral contraceptives can cause liver

damage and jaundice has stipulated it. Hepatitis is another problem that can raise AST and ALT levels. Low levels of AST and ALT may indicate deficiency of vitamin B6. Brenner et al. (1977) found that when different doses of norgestrel were administered to women, the serum levels of levonorgestrel were related to the dosage. Peak serum levels were found 0.5 to 3 hours after oral administration, followed by a rapid, sharp decline. However, 24 hours after ingestion, 20 to 25% of the peak level of levonorgestrel was still present in the serum (Mishell *et al*., 1976).

The various modifications in chemical structure of the different synthetic progestins and estrogens affect their biologic activity. Thus, one cannot judge the pharmacologic activity of the progestin or estrogen in a particular contraceptive steroid formulation only by the amount of steroid present. The biologic activity of each steroid also has to be considered. Using established tests for progestational activity in animals, it has been found that a given weight of norgestrel is several times more potent than the same weight of norethindrone. Studies in humans, using delay of menses or endometrial histologic alterations such as subnuclear vacuolization as end points, also conclude that norgestrel is several times more potent than the same weight of norethindrone (Swyer, 1982).

Norethindrone acetate and ethynodiol diacetate are metabolized in the body to norethindrone. From this study, it has been observed that most women do not undergo liver function test as part of their medical examination before these medicines are prescribed to them. It is suggested that a thorough medical examination involving liver function tests must be undergone before taking them. It is also recommended that checkup be done every six months. Nevertheless, the full implications of continued administration of eostrogen and progestogen for many years on liver proteins are not yet known (Kaunitz, 2004; Hargreaves, 1969;Sy *et al*., 1986).

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References

1. Brenner PF, Mishell DR Jr and Stanezyk FZ (1977) Serum levels of D-norgestrel, luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone in women during and following ingestin of combination oral contraceptive containing dl-norgestrel. *Am. J. Obstet. Gynecol.* 129,133.
2. Briggs M (1980) Effects of oral contraceptive agents on vitamin and mineral requirements. *J. Am. Diet Assoc.* 5, 160.
3. CHPE, Division of Reproductive Health (1984) Family planning methods and practice. US public health service. Department of Health and Human Services, Atlanta, Georgia 30333. USA.
4. Devlin MT (1992) A textbook of Biochemistry with clinical correlation 3rd ed. John Wiley and sons publication USA.pp: 100.
5. Grimes DA, Mishell DR Jr and Speroff L (1993) Contraceptive choices for women with medical problems. *Am. J. Obstet. Gynecol.* 198, 625-630.
6. Hargreaves T (1969) Oral contraceptives and liver function. *J. Clin. Pathol. Suppl.* 3, 1-10.
7. Kaunitz AM (2004) Enhancing oral contraceptive success: the potential of new formulations. *Am. J. Obstet. Gynecol.* 190 (4), 23-29.
8. Kay CR, Crombie DL, Kuenssberg EV, Pinsent RJFH, Richards B, Smith A, Crowther CH (1974) Oral contraceptives and health. The royal college of general practitioners study. *Am. J. Obstet. Gynecol.* 10,150.
9. Kuhl H and Goethe JW (1990) Pharmacokinetics of oral contraceptives, steroids and drug interaction. *Am. J. Obstetric & Gynaecol.* 163, 2113.
10. Mishell DR Jr, Stanezyk FZ and Hiroi M (1976) Steroid contraception. In: Crosignami PC, Mishell DR Jr (eds): Ovulation in the Human. London, Academic press. pp: 10.
11. Ohno H, Tojo H, Kajimura T and Nomura M (1994) Increased serum alkaline phosphatase induced by DT-5061, an oral contraceptive, in rats. *J. Toxicol. Sci.* 19 (3), 507-518.
12. Reitman S and Frankel S (1957) In vitro determination of Glutamic-Oxaloacetic Transaminase (GOT) and Glutamic-Pyruvic transaminase (GPT) in serum. *Am. J. Clin. Path.* 28,56.
13. Skouby SO and Jesperson J (1990) Oral contraceptives in the nineties, metabolic aspects, facts and fiction. *Am. J. Obstet Gynecol.* 163, 276.

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14. Swyer GIM (1982) Petency of pregestogens in oral contraceptives-further delay of menses data. *Contraception.* 26,23.

15. Sy FS, Osteria TS, Opiniano V and Gler S (1986) Effects of oral contraceptives on liver function tests of women with schistosomiasis in the Philippines. *J. Contraception.* 34(3), 283 -294.