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RESEARCH PAPER

THE EFFECT OF STEROIDAL CONTRACEPTIVES ON LIVER ENZYMES AND SERUM PROTEINS IN WHITE ALBINO RATS

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ABSTRACT

This study assessed the influence of steroidal contraceptives on liver enzymes and serum total protein using 48 adult female rats in four groups -A as control and B, C and D as tests. The animals were further divided into two subgroups - *treatment* (A1 - D1; n=6 each) and *reversal* (A2 - D2; n=6 each). Groups A1&A2 received normal feed and water only, while B1&B2, C1&C2 and D1&D2, respectively received 0.03mg/kg of *Norethisterone enantate* intramuscularly once in 6 days for 3 estrous cycles; 0.03mg/kg of *Lynesternol* daily for 6 days and repeatedly for 3 estrous cycles; and a combination of 0.03mg/kg of *Ethinylestradiol* and 0.03mg of *Levonorgestrel* daily for 6 days and repeatedly for 3 estrous cycles. At the end of the *treatment phase*, blood samples were collected from groups A1 - D1 for laboratory analysis, while the treatments for groups B2 - D2, were suspended and monitored for same period (*reversal phase*) prior to blood sample collection and analysis. The results affirmed that steroidal contraceptives had capacity to induce significant elevation and reduction in levels of liver enzymes and serum total protein respectively; hence the call for caution to avoid unwarranted complications.

Key words: **Liver enzymes, Serum, Steroidal Contraceptives,** T**otal protein**

INTRODUCTIION

Insinuations that many women stop using contraceptives due to issues associated with weight gain, water retention, hypertension and venous and arterial cardiovascular complications (Gate and Stone, 1992, Burkman *et al*., 2001; Kemmeran *et al*., 2002; Baillargeon *et al*., 2005a, b) has become a source of worry. Other minor but common side effects of steroidal contraceptives severe enough to cause the discontinuation of its usage, have also been identified to include nausea, breast tenderness, irregular menstrual bleeding and thrombosis (ACOG Practice Bulletin, 2006). Nevertheless, the last 30 years has witnessed the development of formulations with decreasing doses of estrogen and progestin and their use have been associated with very low pregnancy rates akin to those with higher doses of steroids (Vessey *et al*., 2010). Although the use of contraceptives has tremendously reduced unsafe abortion and maternal/neonatal death rates by 25% (Olise, 2011), there is still the need to examine the long term effects of its usage on vital organs of the body such as the liver-being the principal organ for maintaining the internal environment of the body (Aashish *et al*.,2012).

Hargreaves (1969) had several years ago, stated that the liver plays a central role in the metabolism of contraceptives and it has become obvious that these substances can act directly or indirectly on the liver to produce a variety of biological effects; some of which have both physiological and pathological significance. He added also that oral contraceptives can induce liver damage, jaundice and hepatic abnormalities (Hargreaves, 1969). Similarly, there are documented facts that the long term usage of oral contraceptive (OCS) has been associated with altered immunity

leading to increased vulnerability to respiratory, digestive, urogenital and musculoskeletal system diseases (Swain, 1991) as well as blood coagulation disorders (Chadwick, *et al.,* 2002), jaundice, deep vein thrombosis, thromboembolic disease and gallbladder disease (Fernandez and Kaplowitz, 2005). This study therefore, assesses the influence of steroidal contraceptives on liver enzymes and serum total proteins in female white albino Wistar rats.

MATERIALS AND METHODS

Experimental Animals/Grouping: Forty-eight (48) adult female Wistar rats were used for this study. They were obtained from the laboratory animal farm of Anthonio Research Center, Ekpoma, Edo State, Nigeria, and transported to the experimental site where the animals were housed in a well-ventilated room under 12/12 hours light/dark cycle and fed with growers mash produced by Grand Cereals Ltd -a subsidiary of UAO Nigeria PLC, Jos, Plateau State. Water was given *ad libitum*. The animals were divided into four groups (A, B, C and D). Group A served as control, while B, C and D served as the test groups. The animals in each of the groups were further divided into two subgroups - *treatment* (A1 - D1; n=6 each) and *reversal* (A2 - D2; n=6 each) groups.

Drugs of study/Administration: The drugs of study were *Norethisterone enantate*, *Lynesternol*, *Ethinylestradiol* and *Levonorgestrel*. Groups A1&A2 received feed and water only, while B1&B2, C1&C2 and D1&D2, respectively received 0.03mg/kg of *Norethisterone enantate* intramuscularly once in 6 days for 3 estrous cycles; 0.03mg/kg of *Lynesternol* daily for 6 days and repeatedly for 3 estrous cycles; and a combination of 0.03mg/kg of *Ethinylestradiol* and 0.03mg of *Levonorgestrel* daily for 6 days and repeatedly for 3 estrous cycles. The drugs were administered according to the procedures described by Ekhator and Osifo (2012).

Sample Collection: At the end of each of experimental phases –*treatment and* reversal, blood samples were collected from each of the rats via a cardiac puncture using 5ml syringe and needle under diethyl ether anesthesia.

Sample Analysis: The levels Aspartate Transaminase **(**AST), Alkaline Phosphataes (ALP) and Alanine Transaminase (ALT) were determined according to procedures described by Olomo (2012), while Serum Total Protein was determined according to procedures described by Yolton *et al*. (1994).

Data Analysis: Data analysis was performed using the Scientific Package of Social Sciences **(**SPSS version 19) software. The significance level was set at $p<0.05$.

RESULTS

As compared to the control values, the different steroidal contraceptives were observed to have induced significant elevations ($p<0.05$) in ALP, AST and ALT levels for groups B(1), C(1) and D(1), but their withdrawal in the reversal phase induced reductions ($p<0.05$) in the ALP, AST and ALT values for groups B(2), C(2) and D(2), as compared to groups B(1), C(1) and D(1); being statistically significant in ALP values for all the groups, but the AST values for groups B(2) and D(2) (*see* table 1).

Table 1: Effects of Different Steroidal Contraceptives on Liver Enzymes and Serum Total Protein

Liver enzymes	Subgroups	Group			
		A	B	C	D
ALP (u/l)	1	92.00±11.88	161.33±31.06*	212.97±11.90*	53.33±36.86*
	2	92.70±12.47	142.08±28.68*	186.92±31.83*	42.32±36.75*
ALT (u/l)	1	46.92±17.27	50.09±8.81	66.36±33.33	58.71±40.48
	2	46.43±4.86	40.85±18.93	47.66±9.31	40.97±19.82
AST (u/l)	1	120.93±1.39	163.85±43.02	160.35±35.02	148.15±5.94
	2	114.49±4.93	133.67±8.83*	153.06±18.58	121.42±22.59*
Serum total protein (g/dl)	1	9.47±0.71	6.55±0.63	5.62±0.85	5.65±0.10
	2	9.91± 0.10	9.17±1.31	10.16±0.93	6.39±1.06

Key: 1 = Treatment phase; 2 = Reversal (withdrawal) phase; *mean difference is significant at $p<0.05$.
All the values are expressed as mean ± standard deviation.

The results on Serum total protein showed that the administration of steroidal contraceptives led to reductions in total protein levels in test groups B, C and D, compared to the control, but the observed differences were not statistically significant. Interestingly, the reversal phase results showed elevations in total protein levels in group B, C and D, when compared with their values at the end of the treatment phase. However, the values remained lower than the control groups in B and D, except group C with slightly higher total protein level than the control (*see* table 2). The differences in the reversal phase were not statistically significant.

DISCUSSION

The result of this study does affirm the capacity of steroidal contraceptives to induce elevations in serum liver enzymes irrespective of the type administered to the animals. The observed elevations were in line with the reports by Hargreaves e*t al.* (2013), Swain (1991) and Rickenlund *et al.* (2004), but the changes in Serum Total Protein contradicted the finding by Lonnerdal *et al*. (1980) who had reported a significant elevation in the concentration of total protein in women on long term oral contraceptives. Nevertheless, a brief communication by Huisveld *et al.* (1987) had indicated the potential influence of contraceptives on protein metabolism as reflected by an observed capacity to reduced total protein S but not free protein S.

Attention can also be drawn to the report by Duvillard *et al*. (2010) that estrogen-containing oral contraceptives has the capacity to cause elevations in the levels of very low density lipoprotein, but reduction in the levels of plasma high density lipoprotein. These shades of opinion, irrespective of the divide, suggests that the findings highlight contraceptives' capacity to alter vital metabolic processes either negatively or positively, and in a manner that seemingly remain unpredictable due to various factor variations –physiologic, environmental and/or possibly pathologic. In one instance, Adesiyan *et al*. (2011) opined that the use of hormonal contraceptives could negatively impact on infants thriving on exclusive breast feeding after having monitored the capacity of contraceptives to induce significant reductions in breast milk glucose in lactating mothers; exemplifying further, the dimensions of the side effects of contraceptive usage.

Undoubtedly, the observed elevations in the transaminases portrayed a classical case of 'drug induced hepatotoxicity' which, according to Aashish *et al*. (2012), has been implicated in 5% of all hospital admissions and 50% of all acute liver failures. Such elevations simply depict hepatocelluar death (Amacher, 1998; Edoardo, 2005) and/or fatty degeneration in particular (Amacher, 1998); though it can also be used in conjunction with other serum enzymes, in distinguishing non-hepatocellular injury (Amacher, 1998). Similarly, the observed reduction in the serum total protein levels accentuate the capacity of contraceptives to induce liver disease and thus, suggests that long term administration of steroidal contraceptives may be deleterious to the liver. In fact, the reversal stage changes did signify that early withdrawals following monitored symptoms of contraceptive usage can help restore normalcy if an irreversible damage hasn't occurred. Thus, to avoid unwarranted complications, the need for caution in the use of contraceptives cannot be overemphasized.

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AUTHOR'S CONTRIBUTIONS

The work was designed and supervised by Nwangwa, E. and Iyomon, P., played significant roles in laboratory studies, data collection/analysis, and the drafting of the manuscript. Iyomon, P., Nwangwa, E. and Edebiri, O.E. participated in editing and reviewing processes.