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Effect of Oral Contraceptive Pills (Containing Low Doses of Synthetic Hormones) on Liver Function in Adult Female Rabbits

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ABSTRACT

Earlier investigators have reported early Oral Contraceptives Pills (OCPs) to be hepatotoxic. Despite the modifications on early OCPs in term of content and dosage to lessen their side effects, paucity of literatures existed on the effects of newer OCPs on liver function and integrity. This study is designed to investigate the effect of OCP containing low doses of synthetic hormones on liver function and integrity. The study involves 15 adult female rabbits, divided into three groups (A, B and C). Group A serves as the control, while B and C served as the test groups involving lower and higher weight rabbits respectively. Throughout the period of the study water, rabbit feed and grasses were given *ad libitum* to animals in each group. In addition, animals in group B and C received for 7 and 14 days; 0.14 and 0.3 mL kg^{-1} b.wt. of OCP, respectively. At the end of the experiment, blood sample was collected for the analysis of liver function parameters. The results showed progressive increase in all the parameters studied in test group (B and C) compared to control (group A). Specifically, there were significant increases in total bilirubin, AST and ALT between the groups. While group B presented a non significant increase in direct bilirubin, total protein and ALP compared with control values, group C presented significant increase in these parameters compared to control and test group B. The results of this study therefore suggest that OCP regardless of their dosages, have potentials for impairing liver function and may induces liver damage.

Key words: Oral contraceptive pill, liver, biochemical parameters, female, rabbits

INTRODUCTION

Combined hormonal contraceptives have been reported to be safe in women with a range of medical conditions, including well-controlled hypertension, uncomplicated diabetes, Human Immunodeficiency Virus (HIV) infection, anemia and uncomplicated liver disease (ACOG Committee on Practice Bulletins-Gynecology, 2006; WHO, 2010). However, several contraindications to combined hormonal contraceptives have been documented (Petri *et al*., 2005; ACOG Committee on Practice Bulletins-Gynecology, 2006; PDR Staff, 2007; WHO, 2010). The question is what is the effect of combined oral contraceptive on the liver which is reported by Fernandez-Checa and Kaplowitz (2005) to be involved in the metabolism of substances ingested by man? Indeed, according to the WHO (2010), women who have systemic lupus erythematosus with antiphospholipid antibodies or unknown antibody results should avoid using combined hormonal contraceptives.

Worrisome, studies where OCPs have been reported to be hepatotoxic are the finding of early OCPs which are initially marketed with high doses of synthetic hormones. Also recall that the side effects produced by each of these steroids were common and occasionally severe enough to cause discontinuation of their used (Avonts *et al*., 1990; Henderson *et al*., 1991). However, over the years, hormone levels have continually decreased in order to provide formulation with maximum efficiency and minimum side effects (Grimes *et al*., 1993; Liu and Lebrun, 2006). In the past 30 years, many other formulations have been developed and marked with steadily decreasing dosages of both estrogen and progestin components (Okoye *et al*., 2012) and their use have been associated with very low pregnancy rates, similar to those formulations with higher doses of steroid (Shelepova *et al*., 2005).

To the credit of the newer OCP containing lower dosages of synthetic hormones, a study recently reported an anti-obesity potential (Ekhator and Osifo, 2012). Considering therefore the fact that the liver plays a central role in the metabolism of oestrogens and progestogens (Guyton and Hall, 2006), it becomes obvious that these substances can act directly or indirectly on the liver to produce a variety of biological effects which have both physiological and pathological significance. In view of early studies on the hepatotoxic potentials of OCP (which are known to contain high doses oestrogen and progestin), the recent findings on body weight reducing effect of newer OCP (Ekhator and Osifo, 2012) necessitated this study. In addition, despite the modifications on newer OCPs, paucity of information is available on the literature concerning the hepatoprotective/hepatotoxic effect of these newer low dosages of estrogen and progestin OCP. Hence, this study aimed at determining the effect of low doses estrogen and progestin OCP on some hepatic biomarkers of liver function and integrity significance using adult female rabbits.

MATERIALS AND METHODS

Experimental animals: Fifteen adult female rabbits were obtained from Aduwawa market in Benin City, Nigeria and transported to the experiment site where they were housed in a well ventilated room under a 12/12 h light/dark cycle and fed feed (Grower pelletsed produced by Grand Cereals Ltd, a subsidiary of UAO Nigeria PLC, Jos, Plateau State), grasses and water *ad libitum*.

Drug of study: OCP (AVA containing levonorgestrel 0.15 mg and ethinylestradiol 0.03 mg) was purchased from a Medical Pharmacy in Ekpoma, Nigeria. The OCP use consists of 21 hormonal tablets and 7 non-hormonal tablets. Each white hormonal tablet contains a small amount of two different female hormones. These are levonorgestrel (a progestogen) and ethinylestradiol (an estrogen). Because of the small amount of hormones, it is considered as a combined low-dose oral contraceptive preparation.

Experimental grouping: The rabbits were divided into three groups (A, Band C) of 5 rabbits each; A served as the control, while B (low weight group) and C (high weight group) served as the test groups.

Drug administration: Each day a tablet is dissolved in 100 mL distilled water and the appropriate dose per kg was measured out using a 2 mL syringe for oral administration via an oro-gastric tube. Group B received 0.14 mL while group C received 0.30 mL of the prepared drug. These doses were determined based on comparative dosage per body weight proportion akin to humans.

Sample collection: Twenty-four hours after the last administration of OCP (7 days for rabbits in group B and 14 days for rabbits in group C), blood samples were collected from each of the rabbits by means of a cardiac puncture using 5 mL hypodermic syringe and needle under chloroform anesthesia.

Sample analysis: Liver functionality was indicated by the serum concentration of total protein, total and direct bilirubin and these were assayed as describe by Tietz (1994) for total protein and Tietz (1995) for bilirubin contents. Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were estimated for liver integrity using Randox reagent kit using 2, 4-dinitrophenylhydrazine substrate (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) activity was determined for billary integrity with the Randox reagent kit using the p-nitrophenylphosphate substrate as described by Bessey *et al.* (1946).

Data analysis: The Mean±Standard deviation was determined and one-way ANOVA analyses of variance were performed using SPSS version 17 software. The significance level was set at $p<0.05$.

RESULTS

The effect on some liver function parameters in rabbits administered low doses OCP are as shown in Table 1. Total bilirubin, direct bilirubin and total protein were higher in test groups than control. Total bilirubin was observed to be increasing significantly between the groups. In particular, test group C presented significant higher ($p<0.05$) levels of direct bilirubin (1.36±0.47 mg dL^{-1}) and total protein (11.55±1.07 g dL^{-1}) compared to test group B (0.29±0.02 mg dL^{-1}; 8.27±0.51 g dL^{-1}) and control (0.17±0.04 mg dL^{-1}; 7.70±0.57 g dL^{-1}).

The effect on some liver and billary enzymes of liver integrity in rabbits administered low doses OCP are as shown in Table 2. AST, ALT and ALP followed similar pattern of progressive increase within the groups. AST and ALT were significantly highest in group C (20.50±3.11 and

Table 1: Effect of OCP containing low doses synthetic hormones on liver functional parameters OCP administers

		OCP treated groups	
Liver function parameters	Control (group A)	Group B (0.14 mL)	Group C (0.3 mL)
Total bilirubin (mg dl^{-1})	0.70±0.14^a	1.87±0.15^b	4.18±0.36^c
Direct bilirubin (mg dl^{-1})	0.17±0.04^a	0.29±0.02^a	1.36±0.47^b
Total protein (g dl^{-1})	7.70±0.57^a	8.27±0.51^a	11.55±1.07^b

Values are Mean±Standard deviation and values within each liver function parameters having different superscript are statistically significant at $p<0.05$

Table 2: Effect of OCP containing low doses synthetic hormones on liver and biliary integrity parameters

		OCP treated groups	
Liver and biliary integrity	Control (group A)	Group B (0.14 mL)	Group C (0.3 mL)
AST (U L^{-1})	9.50±2.12^a	15.00±1.73^b	20.50±3.11^c
ALT (U L^{-1})	7.00±1.41^a	16.33±2.51^b	22.75±1.26^c
ALP (U L^{-1})	161.50±64.35^a	196.33±3.51^a	269.00±2.16^b

AST: Aspartate-amino transferase, ALT: Alanine-amino transferase, ALP: Alkaline phosphatase; Values are Mean± Standard deviation and values within each liver and biliary integrity parameters having different superscript are statistically significant at $p<0.05$

22.75±1.26 U L^{-1}, respectively) and higher in group B (l5.00±1.73; 16.33±2.51 U L^{-1}, respectively) compared with the control (9.50±2.12 and 7.00±1.41 U L^{-1}, respectively). Although, ALP activity increases within the groups, that in group B (l96.33±3.51 U L^{-1}) was not significantly higher to that in the control. However, ALP activity was significantly higher in group C (269.00±2.16 U L^{-1}) compared to that in group B (196.33±3.51 U L^{-1}) and control (l61.50±64.35 U L^{-1}).

DISCUSSION

The result on the effect of OCPs on liver functionality indicated via total bilirubin and direct bilirubin demonstrated that administration of low doses of synthetic OCPs may have potential for red blood cells destruction in a dose dependent fashion. This is suggested considering the function of the liver in filtration, storage and metabolism of blood and in the formation and excretion of bile (Guyton and Hall, 2006). Considering the results of the present study, OCP containing lower doses of synthetic hormones is similar to older OCPs which contains higher doses of synthetic hormones and by implication is hepatotoxic. In accordance to this finding, ethinylestradiol which is much more potent than the naturally occurring estrogens, remains present in the blood for a longer duration after administration and has a greater effect on the liver (Kapp, 2009). Moreover, progestogens at high doses (norethindrone acetate and norethisterone enanthate) are metabolized to compounds that may have a small effect on liver function although of a lesser degree than the effects of estrogens (Chu *et al*., 2007). This finding may be supported by the fact that the pills cause breakthrough bleeding and amenorrhea as previously noted (Serfaty, 1992).

In another line of thought, the hyper-proteinaemic effect of OCP indicated by the finding of this study on total protein suggest an impact on the colloid osmotic pressure of plasma, protein metabolism, muscle destruction and transport functions via the protein transporting mechanism through albumin. This effect may be due to the estrogen contained in OCPs which is known to stimulate the hepatic synthesis of several nutrient specific transport proteins. Although, Guyton and Hall (2006) have shown that when the tissues become depleted of protein, the plasma can act as a source for rapid replacement, worrisome is the acute effect of increased plasma proteins in terms of colloid osmotic pressure and plasma transport function. By implication, the findings of this study on bilirubin and total protein suggest a relationship between OCP and liver functionality.

In term of liver cellular integrity, the findings of this study are in line considering the contraindications reported with OCP usage (Petri *et al*., 2005; ACOG Committee on Practice Bulletins-Gynecology, 2006; PDR Staff, 2007; WHO, 2010). Judging by the dose depended increase of serum concentrations of hepatic enzymes in the present study, one can emphasize that in a dose dependent manner, OCPs induces alterations in liver cellular integrity as well as the billary tract. This assertion is sequel to the significance of serum concentrations of hepatic enzymes and that these markers leak into the circulation when there is necrosis or damage to the hepatic cells (Oze *et al*., 2010; Murrey *et al*., 2000).

Although, ALT and AST concentrations are known to be high in the liver and wide variety of tissues like muscles and neuronal cells (Bush, 1991; Dial, 1995), the present findings in this regards reaffirms the fact that OCPs have effect to a number of metabolic and nutritional processes. As such, may be the results of the observed significant increase in liver proteins, bilirubin and enzymes. Thus, the observed significant changes as shown by the result of this study, suggests that ingestion of OCPs can induce alterations in the serum liver proteins and concentrations of liver and billary enzymes and such elevations in experimental animals may reflect liver, heart and muscular toxicity.

Specifically, however, AST is known to be more specific for the liver tissue (Bush, 1991; Dial, 1995) and hence, the present findings suggest liver cells' toxicity with low doses of OCPs containing synthetic hormones. Furthermore, the increase in AST and ALP activity could be ascribed to cellular degeneration of myocardial, neuronal and liver cells.

In a different line of thought, the reports that OCPs caused significant lipid peroxidation (Pincemail *et al*., 2007) and oxidative stress (De Groote *et al*., 2009) may be the resultant increase in liver enzymes and proteins observed in the present study considering the implication of oxidative stress in health and diseases. Not unexpected, every drug has been reported to be associated with hepatotoxicity, almost certainly due to its ability to generate free radicals and to cause disturbance in hepatocyte biochemistry (Fernandez-Checa and Kaplowitz, 2005).

Indeed, judging by the findings of this study, the observed results on the hepatotoxic nature of OCPs appears to have challenged the normal physiological function and integrity of the liver. Therefore, ingestion of even low doses of synthetic OCP may affect the liver functionality, cellular integrity and billary system in a dosage dependent manner. Hence, OCP which is mainly used as a birth control may also have potentials adverse effect on the liver function, integrity and billary tract integrity considering the significant high levels of the proteins and enzymes here in study. Base on the observed changes in liver proteins and enzymes, it is therefore suggested that; OCPs administration to patient with liver problems be done with care. On the liver, there is need to further investigates other liver biochemical parameters and most importantly, the histology of the liver in relation to OCPs.

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