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HORMONAL CONTRACEPTIVES AND FRUCTOSE DIET REDUCES HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN BLOOD LIPID ESTIMATION IN FEMALE WISTAR RATS

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ABSTRACT

The study evaluated effects of ethinyl estradiol and norgestrel combined hormonal oral contraceptives doses on blood lipids in fructose fed Female Wistar Rat. Female Rats weighing 94-262g were randomly grouped into four (4) experimental groups (n=6). Prepared 0.15 and 0.3 ml, Duofem combined ethinlyl estradiol and norgestrel oral contraceptives were administered orally (p.o) to fructose fed group A and B animals respectively, while group C animals were fed fructose diet only and the control group D animals were fed normal feed, the procedure lasted for 28 days, twenty four (24) hours after the last treatment, the animals were euthuanized by cervical dislocation, blood samples collected, and lipids profile (total Cholesterol (TCL), triglyceride (TG), High Density Lipoprotein Cholesterol (HDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) were estimated. A quantity, 0.3ml ethinlyl estradiol and norgestrel significantly ($p<0.05$) reduced mean HDL-C level (46.12±2.57 mg/dl) of group A animals compared to control group, on normal diet with mean HDL level of 65.74±3.58mg/dl. Duofem COC produce more severe alteration of lipid homeostasis in Rats on higher combined oral contraceptives (0.3ml) treatment, as a result of insulin-resistance that resulted from chronic fructose consumption.

Keywords: Contraceptives, Fructose, Blood Lipids, Female, Wistar Rats

INTRODUCTION

Hormonal Contraceptives pills may consist of a combination of oestrogen and progesterone commonly known as combined oral contraceptives (COC) pills or progesterone only pills. Combined hormonal pills with oestrogen and progesterone components are most prescribed (Cooper et al. 2022). Ethinyl estradiol, is the low –dose oestrogen component in combined hormonal contraceptives while progestin components may include norgestrel, norethindrone, levonorgestrel and norethinodrel. Hormonal based oral contraception is reliable, convenient, relatively safe and well tolerated among others (Klipping et al. 2021).

Metabolic effects of combined hormonal contraceptives on lipid and lipoprotein differ with the progesterone type and dose (Bastianelli et al. 2017), 35mcg of ethinyl estradiol and relatively lower dose of norgestrel is the most adopted oestrogen and progestin-based contraceptive (Cooper et al. 2022). Progesterone only oral contraceptives have minimal effects on lipids and Lipoprotein, although there is tendency to decrease High Density Lipoprotein (HDL-C) at higher dose (Liebeskind et al. 2022). High dose of ethinyl estradiol may affect lipid homeostasis which is predisposing to cardiovascular diseases. Reducing the doses of both oestrogen and progesterone is associated with relative decrease in the risks associated with hormonal contraceptives pills usage (ACOG, 2006).

Fructose form dietary intakes sourced in fruits, corn syrup, cane or beet and honey. Fructose intake was recommended due to its low glycaemic index, however chronically high consumption of fructose, resulted to impaired insulin sensitivity in rodents (Bantle, 2006). Hepatic lipogenesis and lipotoxicity are believed to play a pivotal role in the metabolic effects of fructose on lipid metabolism (Softic et al. 2016). The consumption of fructose has been associated with increased

hepatic lipogenesis, contributing to the synthesis of fatty acid within the liver. Lipotoxicity, a condition characterized by the adverse effects of altered lipid homeostasis is implicated in various metabolic disorders including insulin resistance (Horst and Serile, 2007). Genetic variability and the dietary habits of human populations and rodents differ, experimental model with rats proved adequate for translational experimental studies (Gill et al. 1989). It is important to note that there is a general similarity between the cardiovascular system of rats and that of other mammals, man inclusive. Despite the difficulty in producing dyslipidaemia and atherosclerosis in rats, special diets may induce an insulin resistant which could result in altered lipid homeostasis that may model dyslipidaemia in rats (Girard et al. 2006).

Blood lipids levels, including cholesterol and triglycerides are commonly assessed in routine blood tests (Nigam, 2011). Special proteins produced in the liver transport these lipids in the bloodstream. The primary protein-bound cholesterol types are Low Density Lipoprotein (LDL-C), known as "bad "cholesterol and High Density Lipoprotein (HDL-C), recognized as "good" cholesterol. The rise in cardiovascular disease incidence correlates with elevated LDL-C and diminished HDL-C concentration (Jung et al. 2022). LDL-C particles are taken up by receptors cells in the liver and scavengers cells –white blood cells embedded in blood vessels, hindering blood flow. Conversely, HDL collects cholesterol, redistributing it to other lipoproteins for transportation back to the liver, minimizing cholesterol deposition in blood vessels. Both LDL-C and HDL-C serve as independent risk factors for cardiovascular diseases (Boden,2000). Understanding the conditions influencing changes in LDL-C and HDL-C levels throughout life is crucial in establishing their effect on cardiovascular health.

MATERIALS AND METHODS

Animals

Female Wistar rats weighing 94-262g used for the study were obtained from central animal house of Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were initially (for a period of two week) housed in wooden cages in a controlled environment at an ambient room temperature and approximately light: dark cycle of 12:12 with unrestricted access to standard pellet food and water *ad-litium*.

Animal Grouping

Animals were grouped based on treatment received (n=6), Group A animals received 0.15ml COC and Fructose diet, Group B, received 0.3ml COC and Fructose diet, Group C were given fructose diet only and Group D(Control), were on normal diet.

All the experimental procedures are performed in compliance with the institutional (University of Ilorin) Animal Care and Use Research Ethics Committee guidelines. The animals were handled in accordance with the National Institutes of Health (NIH 2021) guidelines for care and use of laboratory animals.

Drugs and Chemicals

Duofem® combined oral contraceptive pills (0.15 mg norgestrel and 0.03 mg ethinylestradiol). Other reagents and chemicals used were of analytical grade and of the purest quality available commercially.

Drug preparation

Two (2) tablets of Combined Oral Contraceptives pill (Duofem®) were dissolved in 30ml distilled water to prepare 0.015mg/kg norgestrel and 0.0015 mg/kg ethinyl estradiol COC and also in 6ml distilled water to prepare 0.15 mg/kg norgestrel and 0.015mg/kg ethinyl estradiol COC respectively.

Study design

0.15ml Duofem COC (0.015mg/kg norgestrel, and 0.0015mg/kg ethinyl estradiol) and 0.3ml Duofem COC (0.15 mg/kg of norgestrel and 0.015mg/kg of ethinyl estradiol) were administered to group A and B animals respectively for two (2) weeks. Fructose was added to the feeds of animals is group A, B, and C, from the beginning of third week till the last day of week four, co-administered with COC in group A and B animals. 50g of Fructose measured using weighing balance was added to 150g of the animal feed to make fructose diet given to animals in group A, B and C. Group D animals received normal animal feed for the duration treatment. Twenty-four (24)

hours after the last treatment the animals were sacrificed by cervical dislocation and blood sample collected.

Estimation of Blood Lipids

Blood samples were centrifuged for 10mins at 3,000rd/ min , serum separated from each sample were analyzed for lipid parameters as described by Friedewald et al. 1972: total cholesterol, Triglycerides, HDL-C, A typical procedure used by NHANES 2004.

LDL was calculated. According to Friedewald's equation:

$$[LDL] = \frac{[\text{Total cholesterol}] - [HDL] - [\text{Triglycerides}]}{5}$$

Statistical Analysis

Data were expressed as Mean ± S.E.M and were analyzed using one–way analysis of variance (ANOVA) and post hoc tests (Student's Newman-Keuls) for the multiple comparisons where appropriate using GraphPad InStat® Biostatistics software. The level of significant was set at $p< 0.05$.

RESULTS

Effect of COC and Fructose diet on Total Cholesterol

Animals that received doses of COC (Duofem) co-administered with fructose diet and animals on fructose diet only, showed no significant ($p>0.05$) difference in Total Cholesterol level compare to the control group.

Table 1. Mean and SEM of total cholesterol concentration in each group (n=6).

S/N	Animal grouping	Total Cholesterol (mg/dl)
1	A- 0.15ml COC + Fructose	198. 14 ± 9.10[#]
2	B- 0.3ml COC + Fructose	173. 98 ± 6.06[#]
3	C-Fructose	203. 18 ± 8.42[#]
4	D-Control	209. 47 ± 9.11[#]

[#]Denote $p>0.05$ as compare to Control

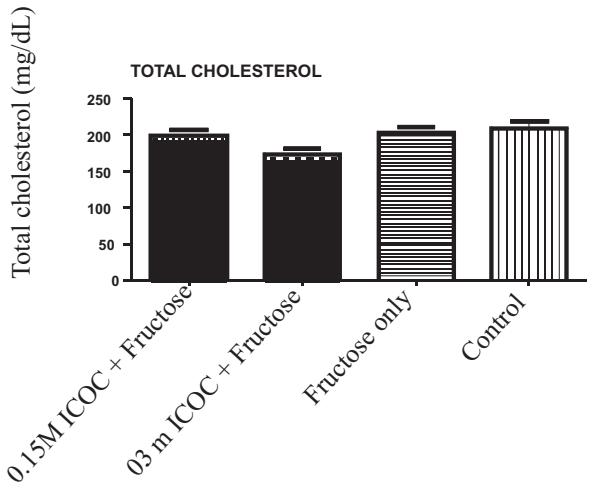

Fig. 1. Effect of COC and Fructose diet on Total Cholesterol

Effect of COC and Fructose diet on Triglyceride

Animals that received doses of COC (Duofem) co-administered with fructose diet and those on fructose diet only showed no significant ($p>0.05$) difference in triglyceride level compare to the control group.

Table 2. Mean and SEM of Triglyceride concentration in each group (n=6).

S/N	Animal grouping	Triglyceride (mg/dl)
1	A- 0.15ml COC + Fructose	66. 33 ± 1.54[#]
2	B- 0.3 ml COC + Fructose	69. 40 ± 0.87[#]
3	C-Fructose	64. 64 ± 1.74[#]
4	D-Control	64. 55 ± 1.39[#]

[#]Denote $p>0.05$ as compare to Control

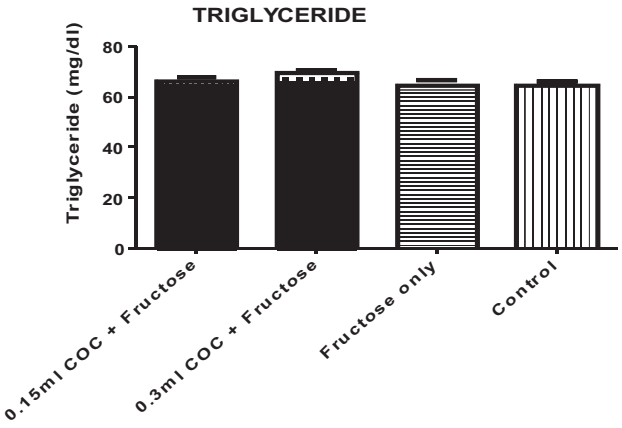

Fig. 2. Effect of COC and Fructose diet on Triglyceride

3. **Effect of COC and Fructose diet on HDL-C**

HDL-C concentration is significantly ($p<0.05$) reduced in animals that received 0.3ml COC (Duofem) and fructose diet compare to the control group.

Table 3. Mean and SEM of HDL-C concentration in each group (n=6)

S/N	Animal grouping	HDL -C (mg/dl)
1	A - 0.15ml COC + Fructose	62. 19 ± 3.58#
2	B - 0.3 ml COC + Fructose	46. 16 ± 2.57*
3	C - Fructose	61. 32 ± 1.43#
4	D - Control	64. 65 ± 3.58 #

#Denote $p>0.05$ as compare to Control
* Denote $p<0.05$ as compare to Control

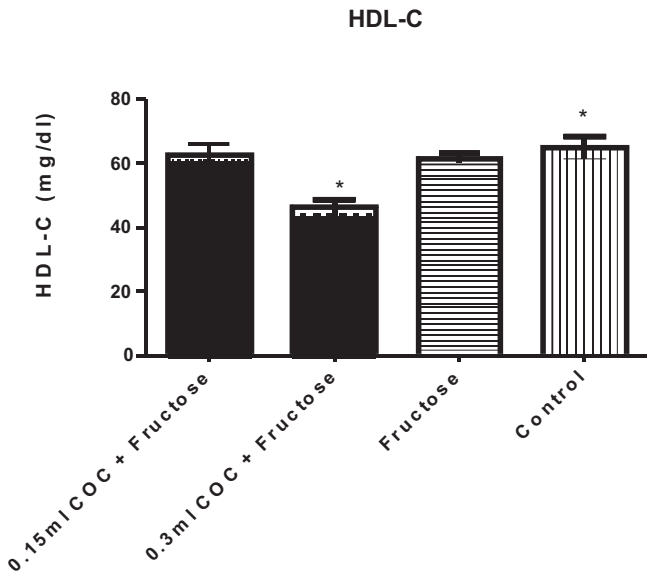

Fig. 3. Effect of COC and Fructose diet on HDL-C

4. **Effect of COC and Fructose diet in the level of LDL-C**

Blood lipid estimation showed no significant ($p>0.05$) difference in the level of LDL-C in animals received doses of COC (Duofem) co-administered with fructose diet and animals on fructose diet only compare to the control group.

Table 4. Mean and SEM of LDL-C concentration in each group (n=6)

S/N	Animal grouping	LDL -C (mg/dl)
1	A - 0.15ml COC + Fructose	61. 81 ± 3. 77 #
2	B - 0.3ml COC + Fructose	51 . 26 ± 2. 86 #
3	C - Fructose	68. 12 ± 1. 59 #
4	D - Con trol	70 . 04 ± 3. 97 #

#Denote $p>0.05$ as compare to Control

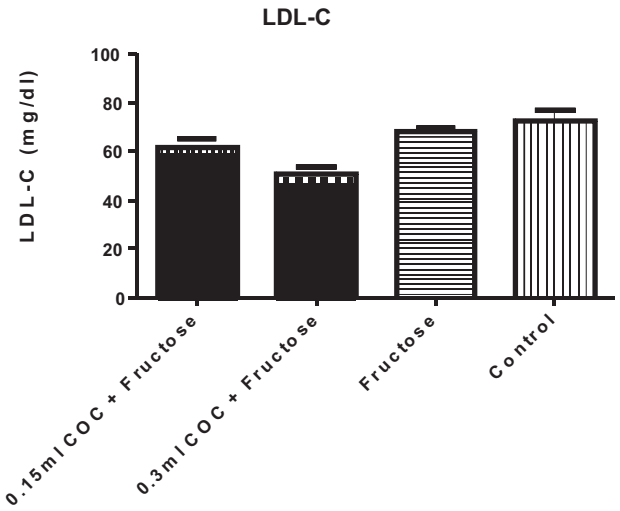

Fig. 4. Effect of COC and Fructose diet on LDL-C

DISCUSSION

Investigating impact of high and low doses of combined hormonal contraceptives on lipid parameters in female Wistar rats fed a fructose-rich diet detail the complications of hormonal contraceptive interactions in the presence of specific dietary conditions. The combined study of ethinyl estradiol and norgestrel COCs along with a fructose mixed feed in rats provide insights into both contraceptive effects and the metabolic consequences of fructose consumption, contributing to a broader understanding of hormonal contraceptives and dietary influences on cardiovascular health (Olatunji et al. 2013). Rats are commonly used in research to understand the impact of dietary components on various physiological parameters (Dwinell, 2010). In this experimental research, the administration of 0.3ml of ethinyl estradiol and norgestrel Combined Hormonal Contraceptives orally resulted in a reduction of HDL-C (high-density lipoprotein cholesterol) in female Wistar rats fed a fructose

diet. However, there was no significant impact observed on Total Cholesterol, Triglycerides, and LDL-C (low-density lipoprotein cholesterol). High doses appear to selectively influence HDL-C levels without significantly altering total cholesterol, triglycerides, or LDL-C. Meanwhile, lower doses exhibit no discernible effects on any of these lipid parameters, when a lower dose of 0.15ml of the same Combined Hormonal Contraceptives was administered, there was no observable effect on lipid parameters in female Wistar rats on a fructose diet. The pharmacokinetics and metabolic pathways of COC in rats were crucial mechanistic considerations. The complexity of these mechanisms underscores the importance of dosage considerations and suggests that the interplay between hormonal contraceptives and fructose diet may have differential effects on specific components of lipid metabolism. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents (Lee et al. 2020).Progesterone component of the contraceptive pill used have no significant effect on Total cholesterol and triglyceride, base on the progestin type, dose administered and route of administration. The administration of ethinyl estradiol and norgestrel, commonly found in combined oral contraceptives (COCs) work synergistically, depending on the specific formulation and dosage of hormonal contraceptives (Sun et al. 2014).

These findings suggest a dose-dependent influence of ethinyl estradiol and norgestrel on lipid metabolism in the context of a fructose-rich diet in female Wistar rats.

CONCLUSION

In conclusion, the experimental research findings suggest that the combination of 0.3 ml of ethinyl estradiol and norgestrel in hormonal contraceptives, when coupled with a fructose-rich diet, leads to a reduction in HDL-C levels. Interestingly, no significant impact was observed on other blood lipid parameters. These results underscore the importance of considering diet and specific hormonal formulations in understanding their nuanced effects on lipid profiles. Further exploration into the underlying mechanisms and long-term implications of these interactions is warranted to enhance our comprehension of the interplay between hormonal contraceptives, dietary factors, and cardiovascular health.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ETHICAL CONSIDERATION

All procedures in this study were performed in compliance with the World Medical Association (WMA Declaration of Helsinki) guidelines in the use of animals in biomedical research re-affirmed by the WMA Council session, Buenos Aires, Argentina, 2016 (World Medical Association 2016).

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