Original Research Article

EVALUATION OF CARDIOVASCULAR DISEASE RISK FACTORS IN ADULT FEMALE RATS EXPOSED TO COMBINED ORAL CONTRACEPTIVE PILLS

ABSTRACT

Background: Prolonged daily intake of oral contraceptive pills may result in cardiotoxicity. However, there are conflicting reports on the effects of combined oral contraceptive pills on cardiovascular disease risk factors.

Aim: This study investigated the cardiotoxicity effect of combined oral contraceptive pills in female albino rats, by the estimation of serum cardiovascular diseases risk factors.

Methods: Twenty-four rats were randomly allotted into three groups of eight rat each. Group A (saline water and feed treated; n=8) which served as control group, Group B (0.6 mg/kg body weight ofcombined oral contraceptive pillstreated for 48 days; n=8) and group C (0.6 mg/kg body weight ofcombined oral contraceptive pills treated for 60 days; n=8).After the required weeks of treatment, the animals were sacrificed and blood and heart tissue samples were collected for biochemical and histological analysisusing standardprocedures.

Result: The result showed significant increased body weight gain after the treatment with combined oral contraceptive pills.There was an elevation in serum C-reactive protein, and reduction in Nitric Oxide levels in the combined oral contraceptive pills treated rats. Total cholesterol, low density lipoprotein, triglyceride, cardiac troponin-1, lactate dehydrogenase, creatine kinase-MB and malondialdehyde levels were increased in the combined oral contraceptive pills treated rats compared with control group. Histological result as revealed by the photomicrographs showed degeneration of transverse muscles, vascular congestion and vascular necrosis.

Conclusion: The study has confirmed that combined oral contraceptive pills causes an alteration in cardiovascular disease risk factorsand cytoarchitecture of the heart. Thus, can induces ischemic heart disease.

Keywords: Cardiovasculardisease**,**combined oral contraceptive pills, hyperlipidemia and C-reactive protein

1. INTRODUCTION

Oral contraceptive pills are artificialdrugs that act on the endocrine system of the female reproductionthat permit sexual union but prevent unwanted pregnancy[1]. They are synthetically made from two steroid hormones such as estrogen and progestin naturally produced in the body[2]. They acton the female reproductive system by inhibiting ovulation via the suppression of luteinizing hormone and follicle stimulating hormone[3], or alteration of the uterine lining to limit fetus development, or increasing cervical mucus secretions to impede sperm penetration into the cervix [4](Shulman, 2011).Globally, over one hundred and fifty (150) million women of reproductive age uses oral contraceptive pills, and it is classified as the most widely used form of birth control [5].Oral contraceptive pills come in a variety of formulation and dosage such ascombined oral contraceptive pills which contain ethinyl estradiol (0.03 mg) and levonorgestrel (0.3 mg), and progestogen- only pills which contain only progesterone [6].These synthetic hormones present in oral contraceptive pills have been documented to have several negative effects, including cardiovascular diseases (CVDs) and

cancers such as venous thromboembolism, stroke, atherosclerosis, myocardial infraction, hypertension, breast cancer, endometrial cancer, and ovarian cancer [2, 7].

CVDs are a group of disorders that involves the organs of the cardiovascular system[8]. They are the principal cause of death and disability worldwide, with an estimated annual death rate of 17.9 million people [9].CVDs include; coronary heart disease, stroke, cardiac failure, hypertension, cardiomyopathy, venous thrombosis, congenital heart disease, peripheral artery disease, and thromboembolic diseases [8]. There are several reports on the association between oral contraceptive pills use and incidence of CVDs, but the underlying mechanism is not well established. However, there are few proofs that the mechanism could be linked to the effect of the synthetic steroid hormones (estrogen and progestogen) present in oral contraceptive pills by influencing CVDrisk biomarkers such as plasma lipid levels, nitric oxide, C-reactive protein, and homocysteine [10].The incidence of risk factors for CVDs is on the rise in the developing countriesglobally[11].It is reported that approximately 80% of the global burden of CVD will occur in low and middle-income countries, and CVD account for the majority of deaths due to chronic diseases [12].An increase in plasma lipids (hyperlipidemia), elevated C-reactive protein concentration and decrease in nitric oxide concentration suggests potential risk of CVD development[5].

Hyperlipidemia has been established to increase the risk of development of cardiovascular diseases in human and animals [13]. Estrogen and Progestogen present in combined oral contraceptive pills have been documented to have several impacts on lipid metabolism [14]. The dosage, the hormonal components (ethinyl estradiol and levonorgestrel), and the anti-androgenic effect of progesterone all affect blood lipid levels when COCs are consumed [15]. Results from earlier studies has shown that those who use oral contraceptives had greater serum total cholesterol, triglyceride, low-density lipoprotein levels than people who don't use them [2, 16,17].

C-reactive protein (CRP) is one of the most sensitive acute phase reactants [18], with its levels rising during inflammatory and autoimmune diseases[19]. It plays a significant role in atherogenesis via promoting endothelial dysfunction, activation of complement pathways, lipids uptake by macrophage, and decreasing endothelial nitric oxide production [20]. Previous works have shown a link between combined oral contraceptive pillsuse with an increasein inflammatorybiomarkers (particularly C-Reactive Protein) in healthy women[21]. It has also been reported that approximately one in everythree women of COC users, presentCRP greater than3 mg/L[22].Nitric oxide (NO) is produced from L-arginine in the vascular endothelium and can induce vasodilation, preventing the buildup and movement of smooth muscle cells and inhibits platelet aggregation and migration [23]. Reduced bioavailability nitric oxide is hypothesized to be involved in the onset and development of atherosclerosis[23]. Steroid sex hormones (especially estrogen) have been documented to have effect on endothelial NO production[24].

In recent times, the likelihood of alterations in various metabolic processes causing elevated cardiovascular disease risk biomarkers, by oral contraceptive pills, has received much attention but regrettably, there has been contradictory reports in this regard. Some authors suggest that this discrepancy in reports could be attributed to the chemical heterogeneity of the estrogen family and their varying concentration. Thus, this study was carried out to investigate the influence of combined oral contraceptive pills on cardiovascular disease risk biomarkers in female albino rats.

2.0 MATERIALS AND METHODS

2.1 Experimental Animals

A total of twenty-four adult female rats*(Rattus norvegicus)* weighing between 120g and 210g were purchased from the laboratory animal house of the Department of Pharmacology, College of Health Sciences, Niger Delta University, Wilberforce Island, Nigeria.The rats were housed in typical environmental conditions, which comprised of relative humidity, a 12-hour light/dark cycle, and a

temperature range of 25 ± 30^{O}C.Rats received unlimited accessto water and standard commercial rat diet. After acclimatization for two weeks, the rats were randomly selected into three groups; Group A (saline water and feed treated; n=8) which served as control group, Group B (48days COC-treated; n=8) and group C (60days COC-treated rats).Good Laboratory Practice (GLP) was followed when handling animals, and the National Institutes of Health Guide for Care and Use of Laboratory Animals was followed strictly when conducting the research, and was approved by the approved bythe Research and Ethical Committee of College of Health Sciences, Niger Delta University.

2.2 Protocol

Groups A (control) received 2 ml/kg saline water and normal rat feeds daily for 48 days. Group B received 0.6mg/kg body weight of combined oral contraceptive pills once daily in 5-days cycle (4-day treatment with 1-day break) for 48 days, and Group C ratsreceived 0.6 mg/kg body weight of Combined Oral Contraceptive pills once daily in 5-days cycle (4-day treatment with 1-day break)for 60days. In addition, the body of the animals was monitored by taking the taking the initial body weight before administration of the drugs, and final body weight after the completion of drug administration.

2.3 Preparation of the Drug and Administration

A Levofem® tablet which is a brand of combined oral contraceptive pill made up of 0.03 mg ethinyl estradiol and 0.3mg levonorgestrel, manufactured by PT Harsen, Jakarta Timur-13750 (Indonesia) and imported and distributed by Deep K. Tyagi Foundation Nigeria was used for the study. The drugs were bought from Danson Pharmacy Store Opolo, Yenagoa, Bayelsa State. The concentration of the drug used was adopted from Toryila*et al.*, [25].The drug contains 28 pills; the white tablet contains 0.03mg ethinylestradiol and 0.3mg levonorgestrel and each brown tablet contains 75mg ferrous fumarate. Twenty-one white tablets of the drug were dissolved in 100ml of distilled water. The drug was administered daily by oral gavage syringe. 0.6mg/kg body weight concentration of the drug was administered daily in 5-days cycle (4-day treatment with 1-day break) for forty-eight days. The dose and volume of the drug administered was calculated using the formula.

$$\text{Concentration of drug (mg/ml)} = \frac{\text{Weight of Rat (kg)}}{\text{Volume (ml)}} \times \text{Dose rate (mg/kg)}$$

The drug was administered once daily via oral route in a five days cycle (4 days treatment with 1-day break) using metal cannula attached to 2.0 ml syringe and lasted for a period of 48 days and 60 days.

2.4 Sample Collection and Preparation

After the required weeks of treatment, the rats in each group were reweighed to determine their final body weight and the animals were anaesthetized to death with chloroform. Blood was collected via heart punctures and dispensed into K_3EDTA and plain container. The blood in the plain container was left at room temperature for 60minutes to clot properly and centrifuged at 3000 rpm for 10minutes to obtain serum. The serum sample was used for the estimation of C-reactive protein, nitric oxide, lactate dehydrogenase-1, creatine kinase-MB, Troponin-1, and malondialdehyde, while the plasma sample from the K_3DTA was used for the determination of total cholesterol, high-density lipoprotein, and triglyceride. Thereafter, the abdominal cavity of the animals was opened by midline incision to collect the heart tissue for histological analysis.

2.5 Assessment of Biochemical Parameters

Serum LDH was measured by Kinetic UV method as reported byMarkler and Hinrichs [26] with modification.Serum CK-MB was determined by Kinetic UV method as reported Passing and Bablok [27]with modification. The enzyme lactate Dehydrogenase catalyzes the reduction of pyruvate and NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the LDH activity in the sample. Serum malondialdehyde was determined by

the method of reported by [28] using an auto-analyzer spectrophotometer. Serum C-reactive protein and troponin-1 was estimated using Enzyme Immunosorbent Assay kits (Cat No. MBS2603063 and DEIA217 respectively) was used for the determination of serum cardiac troponin and C-reactive protein as reported by Babuin and Jaffe[29].Fasting plasma total cholesterol, high-density lipoprotein and triglyceride were determined using a standardized enzymatic spectrophotometric method using reagents obtained from Agappe Diagnostics Ltd. (Agappe Hills Dist. Ernakulam, Kerala India). LDL-cholesterol was calculated from the Frielwald`s Formula; LDLcholesterol (mmo/l) = Total cholesterol–triglyceride/5-HDL-cholesterol.

2.6 Histopathological Examination

The excised heart tissue from each rat were cut into slabs of about $6mm^3$ in size and fixed immediately in 10% formal saline. Automatic Tissue Processor- Histokinette (LEICA TP 1020) was used for tissue processing, and all tissues were embedded in paraffin wax in tissue Embedder (LEICA EG 1160). The tissue blocks were sectioned in a Rotary Microtome (Heitz 150 Rotary Microtome) at 5 microns. The slides were stained in Haematoxylin and Eosin using the method described by Ochei and Kolhatkar, [30] for general tissue architecture. The stained slides were examined under a high-resolution microscope (Olympus BX60MF, Japan), and photomicrographs were taken at a magnification of ×400.

2.7 Statistical Analysis

Data were expressed as mean and standard deviation (Mean ± SD). A statistical software for social sciences (SPSS) version 23.0 was used for the statistical group analysis. The Student t-test and one-way analysis of variance (ANOVA) were used to compare the groups. $P<0.05$ was accepted as the statistical significance difference with confidence interval of 95%.

3.0 RESULTS

Table1 showed the initial weight and final weight of female adult albino rats studied after combined oral contraceptive pills administration. Group B and C were administered with 0.6mg/kg body weight of combined oral contraceptive pills except group A which received saline water and rat diet only. The result showed that all the groups (Group A, B & C) showed increased in body weight gain and were statistically significant at $p<0.05$. However, the mean (31.107) and percentage (18.79%) weight gain were higher in the rats treated with COCs for 60days (Group C) when compared to other groups (A & B).

Table 2showed the effect of combined oral contraceptive pills on serum C-reactive protein, Nitric oxideand lipid profile levels of adult female albino rats. There was a statistically significant ($p<0.05$) increase in serum C-reactive protein (CRP) level in the combined oral contraceptive pills treated groups (Group B& C) compared with control (group A) (2.23±0.41, 2.27±0.57 Vs 1.23 ± 0.34). Serum Nitric Oxide level in group B& C were lower than the control (group A) (2.69±0.59, 2.62±0.46 Vs 4.68±0.92) and was statistically significant at $p<0.05$. Furthermore, Lipid profile parameters;total cholesterol (2.23±0.41, 2.27±0.56), triglyceride (4.15±3.28, 4.20±3.58) and low-density lipoprotein (1.24 ± 0.41, 1.37 ± 0.11) showed a statistically significant ($p<0.05$) increase in the Group B& Crespectivelywhen compared with Group A (Control) (0.97 ± 0.08, 1.74 ± 0.55, and 0.26 ± 0.19) respectively. However, High-Density Lipoprotein (2.72±2.0) levels showed a slight reduction in Group B compared with Group A (2.61±1.7), but not statistically significant ($p>0.05$). The result further revealed that mean values of all the analyzed biochemical parameters was higher in the rats administered for 60days (Group C) than those administered for 48days.

Table 3 showed theeffect of administered combined oral contraceptive pills on troponin 1, LDH-1, CK-MB, and Lipid Peroxidation product malondialdehyde of Female Adult Albino Rats Studied. The result revealed that all the cardiac biomarkers analyzed showed upward trend in group B and C(combined

oral contraceptive pills treated group) when compared with control (group A). Troponin-1 was 0.31 ± 0.03, 0.33±0.03 vs 0.11 ± 0.02, Lactate Dehydrogenase was69.36 ± 10.06, 69.48 ± 10.06 vs48.88 ± 11.53, Creatinine Kinase-MB was 13.50±1.06, 13.55±1.06 vs 10.00±1.46, while Malondialdehydewas2.46±0.66, 2.59±0.66 vs 1.20 ± 0.24; and were all statistically significant at p=0.0001 for troponin, and p=0.017 for lactate dehydrogenase, p=0.02 for creatinine kinase MB and p=0.04 for malondialdehyde.

Histological examination of the Haematoxylinand Eosin stained tissue sections showed in Figure 1 revealed that tissue section of the control group (Group A) shows normal morphology of the heart muscles, the transverse section,oblique section, and the central nuclei. Figure 2 shows thephotomicrograph of transverse section of heart of female rat administered with 0.6mg/kg body weight of combined oral contraceptive pills for 48 days. Slide revealed degeneration of transverse muscles (D), vascular congestion (V), and tissue necrosis (N), suggestive of ischemic heart disease. Figure 3 shows the pphotomicrograph of transverse section of heart of female rat administered with 0.6mg/kg-body weight of combined oral contraceptive pills for 60 days. The section shows alteration in normal architecture of the heart with localized area of ischemic necrosis suggestive of obstruction in blood flow.

Table 1:Mean Body Weight of Female Albino Rats treated with Combined Oral Contraceptive Pills

Treatment	**Initial Weight (n = 6)**	**Final Weight (n = 6)**	**Mean Weight Gain**	**% Body Weight Gain**	***t*-statistic**	***p* value**	**Rmk**
	Mean ± SD(g)	Mean ± SD (g)	(g)	(%)			
GRP A (control)	130.50 ± 9.05	160.17 ± 20.93	29.67	17.64	-3.90	.011	S
GRP B (48 days)	161.67 ± 7.12	179.17 ± 8.66	17.50	9.77	-4.50	.006	S
GRP C (60 days)	154.50 ± 4.68	185.67 ± 8.52	31.17	18.79	-9.33	.000	S

Key: SD = Standard deviation, S = Significant, NS = not significant, GRP=Group*;* GRP A= Control (Feed and water).
GRP B = Combined Oral Contraceptive pills administered group (0.6mg/kg body weight) for 48days; Group C= Received 0.6 mg/kg body weight of Combined Oral Contraceptive pills for 60days.

Table 2: Effect of Combined Oral Contraceptive Pills on C-reactive protein, Nitric oxide and Lipid Profile Parameters of Female Adult Albino Rats Studied

Treatments	CRP (mg/dl)	NO (ng/mL)	HDL (mmol/L)	TCHOL (mmol/L)	TRIG (mmol/L)	LDL (mmol/L)
GRP A (control)	1.23 ± 0.34	4.68 ± 0.92	0.43 ± 0.16	0.97 ± 0.41	1.74 ± 0.55	0.26 ± 0.19
GRP B (48 Days)	2.31 ± 0.40	2.69 ± 0.59	0.41 ± 0.17	2.23±0.41	4.15±3.28	1.24 ± 0.01
GRP C(60 Days)	2.27 ± 0.57	2.62 ± 0.46	0.40 ± 0.15	2.27±0.56	4.20±3.58	1.37 ± 0.11
P-value	.002*	.004*	.867	.000*	.047*	.001*

Key: = *Significance difference observed p<0.05.CRP =C- Reactive Protein; NO = Nitric Oxide; HDL = High density Lipoprotein Cholesterol; CHOL=Total Cholesterol; TRI = triglyceride; LDL= Low Density Lipoprotein Cholesterol; GRP A= Control (Feed and water); GRP B = Received 0.6 mg/kg body wt. of Combined Oral Contraceptive pills for 48days; Group C= Received 0.6 mg/kg body wt. of Combined Oral Contraceptive pills for 60days .

Table 3: Effect of Combined Oral Contraceptive Pills on Troponin 1, Lactate Dehydrogenase-1, Creatine kinase-MB, and Malondialdehyde of Female Adult Albino Rats Studied

Treatments (ng/L)	cTn-1 (IU/L)	LDH (IU/L)	CK-MB (nmol/L)	MDA
GRP A (Control)	0.11±0.02	48.88 ± 11.53	10.00±1.46	1.20 ± 0.24
GRP B (48 Days)	0.31±0.03	69.36 ± 10.06	13.50± 1.06	2.46±0.66
GRP C (60 Days)	0.33±0.03	69.48 ± 10.06	13.55± 1.06	2.59±0.66
P-value	.000*	.017*	.002*	.004*

Key: = *Significance difference observed p<0.05; CTn1 = Troponin-1; LDH = Lactate Dehydrogenase; *CK-MB* = Creatinine Kinase-MB;MDA = Malondialdehyde*;* GRP A= Control (Feed and water); GRPB = Received 0.6 mg/kg body wt of Combined Oral Contraceptive Pills for 48days; Group C= Received 0.6 mg/kg body wt. of Combined Oral Contraceptive pills for 60days.

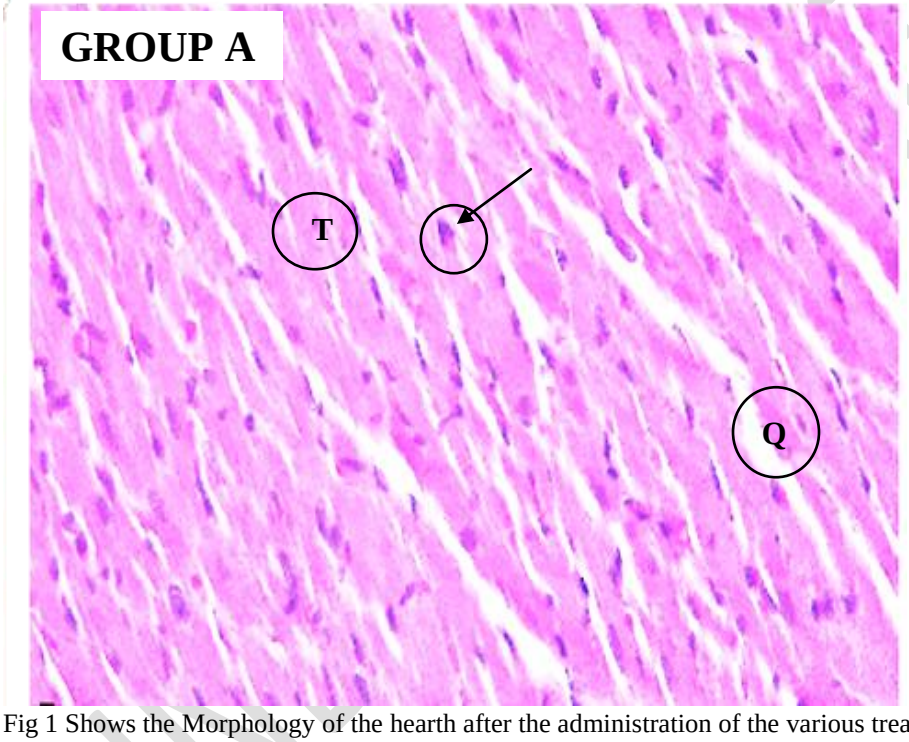

Fig 1 Shows the Morphology of the hearth after the administration of the various treatments for 60 days.
Slide shows normal morphology of the heart muscles, the transverse section (T),oblique section (Q), the central nuclei (arrow) (X40)

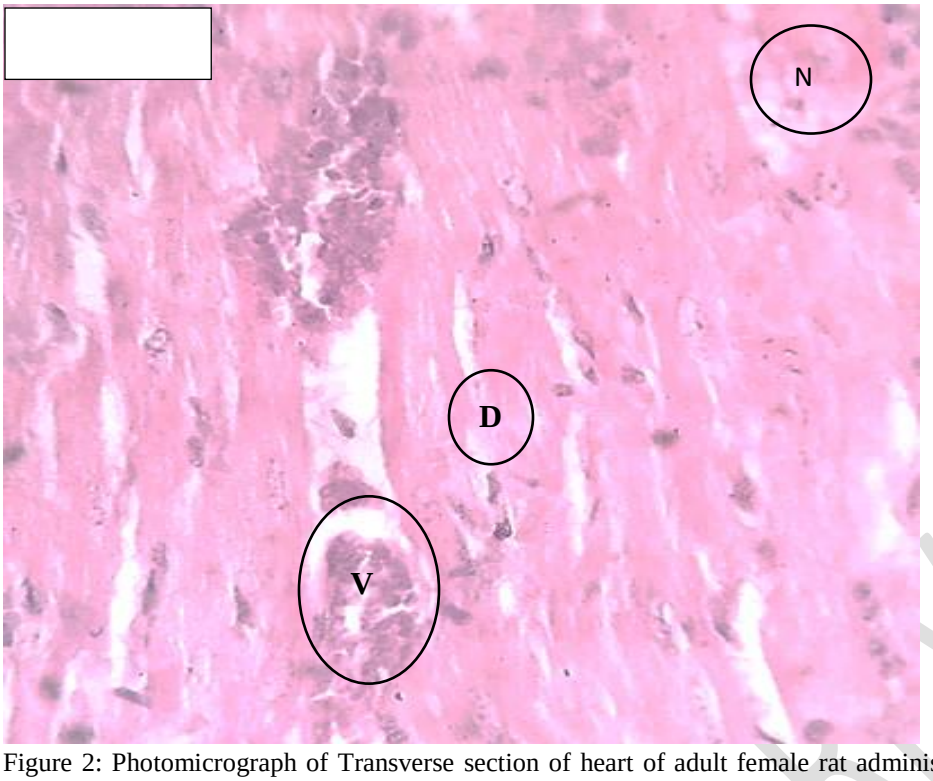

Figure 2: Photomicrograph of Transverse section of heart of adult female rat administered with0.6mg/kg body weight of combined oral contraceptive pills for 48 days. Slide shows degeneration of transverse muscles(D), vascular congestion (V), with areas of necrosis (N)(X40)

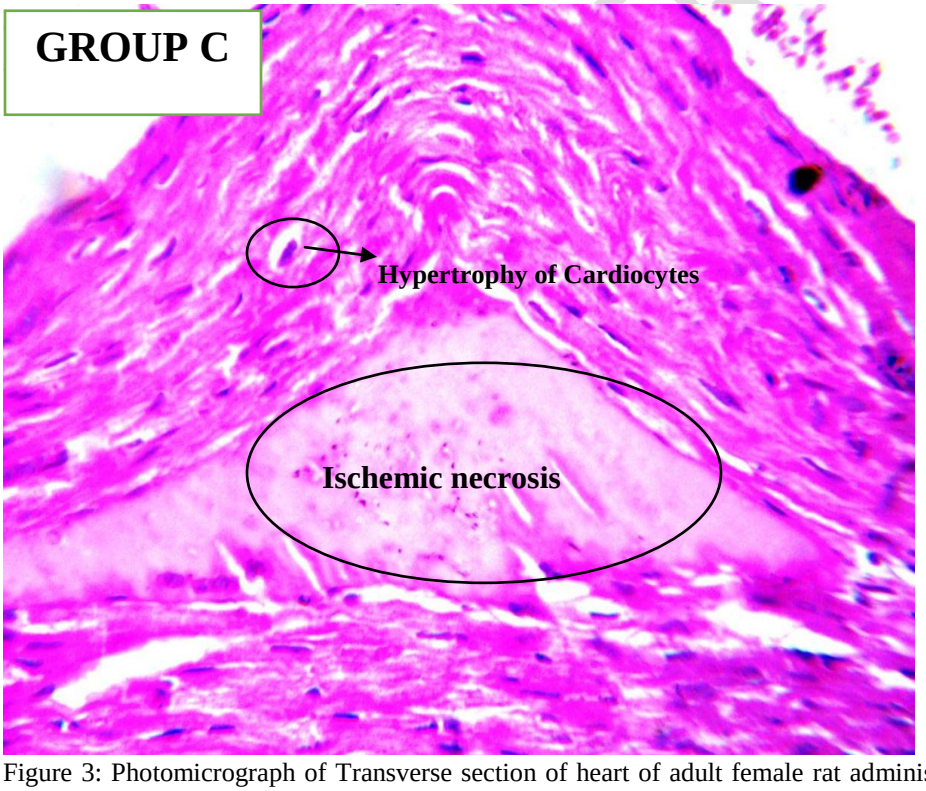

Figure 3: Photomicrograph of Transverse section of heart of adult female rat administered with 0.6mg/kg-body weight of combined oral contraceptive pills orally for 60 days. The section shows alteration in normal morphology of the heart with localized area of ischemic necrosis suggestive of obstruction in blood flow.

4.0 DISCUSSION

If population growth continues at the current rate, it will undoubtedly have an adverse effect on public health and global resource availability. Unfortunately, due to a number of known side effects that have been documented by many authors, contraception, which could have countered this extraordinary growth rate, continues to be inadequately utilized.The current study was carried out to evaluate the concentration of cardiovascular disease risk biomarkers in adult female rats treated with combined oral contraceptive pills.

Previous studies demonstrated that women on hormonal contraceptives containing synthetic steroid hormones (estrogen and progesterone) usually experience changes in body weight [31,32]. In the present study, the initial body weight of the animalsbefore treatment was lower than the final body weight of the combined oral contraceptive pills treated rats and control group. This result suggests that combined oral contraceptive pills caused statistically significant ($p<0.05$) body weight gain. This confirms the works ofAsma *et al.,* [33] and Farnaz *et al.*,[34]; and contradicts the reports by Ekhator and Osifo, [32]. This weight gain observed could be attributed to estrogen which has been reported to cause reduction in postprandial lipid oxidation and increase fat mass[32], while progestin causes increase in appetite and permanent weight gain by the stimulation of renin-angiotensinogen mechanism, increased fluid retention, decreases insulin sensitivity and altered carbohydrate metabolism, and alteration ofbrain metabolism, which would lead to an increase in fluid intake, causing weight gain[32].

Studies have demonstrated that taking combined oral contraceptive pills raises the risk of developing cardiovascular diseases. Hormonal contraceptives contain artificial estrogen and progestogen, which have been shown to directly modulate the transcriptional production of a number of molecules in the liver and have a variety of immunomodulatory effects. This predisposes to thromboembolic events by triggering inflammatory mechanisms [36]. C-reactive protein,as a biomarker of inflammation is one of such moleculesthat has prognostic importance for the development of atherogenesis via the promotion of endothelial dysfunction, activation of complement pathways, lipids uptake by macrophage, and decreasing endothelial nitric oxide production [20]. In this study, the serum C-reactive protein levels of combined oral contraceptive pills treated rats (Group B & C) was significantly ($p<0.05$) elevated when compared with group control (Group A). This could be due to the direct immunomodulatory influence of estrogen on hepatic synthesis of C-reactive protein. This result was in consonance with the reports of the studies by Cauci et al.[37] and Ridker and Silvertown, [18]. However, it is inconsistent with the report by Zahra et al.,[16] which reported a non-significant difference in serum C-reactive protein levels of combined oral contraceptive pills users.

Nitric oxide is producedby L-arginine in the vascular endothelial cells, and it can lead to vasodilatation and impedes the accumulation of platelets and their migration[23].Nitric Oxide levels in the current study was significantly ($p<0.05$)reduced in the combined oral contraceptive pills administered rats (Group B& C) in comparison with the control (Group A). This reduction in nitric oxide may be attributed to the impact of sex hormones (17-α estradiol) on nitric oxide production and inhibition of nitric oxide synthase (NOS) in smooth muscle cells [38]. This confirms the works of Fallah et al. [23], which documented a reduction in nitric oxide level during intake of a combination of ethinylestradiol and levonorgestrel pills.However, it disagrees with Zahra et al.[16], which established a non-significant difference in serum nitric oxide levels of combined oral contraceptive pills users.

Hyperlipidemia has been established to increase the risk of development of cardiovascular diseases in human and animals [13]. Estrogen and Progestogen present in combined oral contraceptive pills have been documented to have several impacts on lipid metabolism [14]. The dosage, the hormonal components (progestogen and ethinylestradiol), and the anti-androgenic effect of progesterone all

affect blood lipid levels when COCs are consumed [15]. Results from earlier studies has shown that those who use oral contraceptives had greater serum cholesterol levels than people who don't use them [16, 17]. In the current study, rats treated with combined oral contraceptive tablets (Group B&C) had significantly higher levels of triglycerides, low density lipoprotein, and total cholesterol ($p<0.05$) than the control group (Group A).High-Density Lipoprotein (HDL) level was slightly lower in combined oral contraceptive pills treated rats (Group B& C), but not significant. The increase in serum lipid levels could be due to the influence of estrogen on lipid metabolism, particularly hepatic lipogenesis resulting in elevated total cholesterol, triglyceride and LDL levels in circulation, which could be linked to higher risk of cardiovascular diseases in the future. This confirms the reports of Zahra *et al*., [16]. It is inconsistent with the report of Kim and park, [39] which reported an increased HDL levels and decreased LDL levels in COC pills users compared with non-users.

Patients with myocardial infarction, acute myocarditis, and atherosclerosis have increased serum/plasma cardiac biomarkers, which are helpful in clinical diagnosis[40]. A number of these cardiovascular illnesseshave been associated with the use of combined oral contraceptives [41]. In the present study, the serum levels of troponin-1, lactate dehydrogenase, and creatine kinase-MB were significantly ($p<0.05$) higher in the combined oral contraceptive pills treated rats (group B& C) than the control (group A). This could be due to the fact that COCs increases cellular lipid peroxidation activities causing the leakage of cardiac proteins into the extracellular fluid, or could be due to elevation of many coagulation factors causing hypercoagulability [42], which is seen to be the most vital determinant of atherosclerosis, [43], which in turn precedes myocardial infarction and ischemic stroke causing increased cardiac markers.

Oxidative stress has been linked to the development and progression of cardiovascular diseases [44] (Mallick *et al*., 2015). The effect of COCs on biotransformation and metabolism is not well elucidated [45], and the nexus between COCs use and oxidative stress remain controversial [46]. However, Pincemail et al.,[47]posited that prolonged use of oral contraceptive pills resulted in increased lipid peroxidationactivities in cells.Malondialdehyde is a clinical indicator of oxidative stress, which is produced as a result of cellular lipid peroxidation[48]. Data fromour study revealed that malondialdehyde levels was significantly ($p<0.05$) elevated in the rats treated with COC pills compared with the control group. This is credence to an increased production of reactive oxygen species in the COC pills administered rats. This demonstrates that combined oral contraceptive pills can induce oxidative stress. This confirms the works of De-Groote *et al*., [46]; Massart*et al.,* [49] and Finco et al., [50], which reported that patients using exogenous hormonal contraceptive pills, have a dramatic increase in free radicals.

Histological examination of the Haematoxylin and Eosin stained tissue sections showed in Figure 1 revealed normal histomorphology of the heart muscles, the transverse section, oblique section, and the central nuclei. Results in figure 2 & 3 shows degeneration of transverse muscles (D), vascular congestion (V), and tissue necrosis (N), suggestive of ischemic heart disease. There was alteration in normal architecture of the heart with localized area of ischemic necrosis suggestive of obstruction in blood flow.These histological results confirmed the result of the biochemical findings of elevated cardiovascular disease risk factors and cardiac biomarkers in the combined oral contraceptive pills treated rats, and the effect is duration dependent.

5.0 CONCLUSION

The results suggest that intake of combined oral contraceptive pills may result in elevation of serum C-reactive protein, reduction innitric oxide levels, increased plasma total cholesterol, low-density lipoprotein, triglyceride levels and elevated cardiac troponin-1, lactate dehydrogenase, and creatine kinase-MB levels, as well as malondialdehyde levels. As these diagnostic markers are known cardiovascular disease risk factors, any alterationin the body is reported to be associated with increased

riskof cardiovascular diseases. The findings revealed a possibleimplication of combined oral contraceptive pills use in the development of cardiovascular diseases.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Research and Ethical Committee of College of Health Sciences, Niger Delta University. Animal handling was carried out according to Good Laboratory Practice (GLP) and in accordance with National Institute of Health Guide for care and Use of Laboratory Animals.

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