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Dynamics of combined oral contraceptive: a study of some haemostatic parameters in female wistar rats

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Abstract

Contraception is an important health issue in preventive medicine because it protects women globally from the effects of unwanted pregnancy and allows them to integrate into society. The mechanism by which COCs predispose these events remains unclear. This research priority included efforts to discover the effect of COC (DUOFEM) on haemostatic parameters and possible mechanism of actions in female wistar rats.Eighty (80) female wistar rats weighing 180-250 g were used for the study. They were divided into four groups of 20 rats each comprising 10 treated and 10 control rats.The treated groups received COC (DUOFEM) 0.6 mg/kg body weight intragastically for 36, 48, 60 and 72 days in 5-day cycles (4-day treatment with a 1-day break).An enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of Protein C and S Antigen in citrated rat plasma. Antithrombin (AT) was determined by Chromogenic Assays.Prothrombin time (PT), Activated partial thromboplastin time (APTT) were performed using Sysmex CA-6000 Coagulation Analyzer. There were significant decreased inprothrombin time (PT), fibrinogen (Fib), antithrombin (AT), protein C (PC) and protein S (PS) in all treated groups compared to controls($P<0.001$).There were however no significant changes in activated thromboplastin time (APTT).This study has challenged the concept that reducing doses of both estrogen and progesterone in COCs may eliminate the risk associated with their use. Even at the lowest concentration of estrogen and progesterone in DUOFEM, the safety of COC is yet to be achieved. COC users should be monitored for haemostatic parameters.

Key words : Combined Oral Contraceptives, Haemostasis, Wistar rats

INTRODUCTION

Oral contraceptives are a simple form of contraception used by women worldwide. The oral contraceptive is one of the greatest and most influential developments of the twentieth century. Combined oral contraceptive (COC) is the most reliable method ofcontraception. COCs are currently among the most commonly used drugs in developed countries; over one hundred million women worldwide are currently using oral contraceptives. Combined oral contraceptive (COCs) pills contain both progestin and estrogen. The COCs are readily available and acceptable in developing countries to reduce population growth and to prevent morbidity and mortality arising from unwanted pregnancies.[1]

Oral contraceptives had been reported to have beneficial effects in reducing the incidence of pelvic inflammatory disease, decrease risk of ectopic pregnancy, benign breast lesions, ovarian and endometrial cancers, protection against osteoporosis and rheumatoid arthritis among the users.[2]Oral contraceptives are sometimes used to treat heavy or irregular menstruation and endometriosis. Oral contraceptive agents can also be used in hormonal replacement therapy, and in the emergency post-coital contraception. Oral contraceptive decreases the risk of ectopic pregnancy, benign breast lesions, ovarian and endometrial cancers, and offer protection against osteoporosis and rheumatoid arthritis.[3]

The World Health organization (WHO) and other global organizations are seeking ways to increase the amount of information and access people have to contraception and other resources related to family planning all round the world.[4]The use of Combined Oral Contraceptive (COC) and hormonal replacement therapy has been linked to increased venous and arterial thrombo-embolism, cardiovascular diseases, cancers and stroke. Past studies suggest that estrogenic hormones significantly affect pro-inflammatory pathways.[5] Whether these changes have clinical significance remains to be determined. Women on combined oestrogen-progestrogen oral contraceptives have repeatedly been reported to exhibit changes in blood coagulation which may account for their elevated risk of thrombo-embolic disease.[6]

Estrogen modifies the haemostasis balance and their use as COC is clearly related to a higher risk of both venous and arterial thrombosis. The mechanisms of action remain to be proved.[7][8] The mechanism by which COCs predispose to thromboembolic events remains unclear. This study investigated whether changes in haemostasis occur after COC administration in female wistar rats

MATERIALS AND METHODS

Eighty (80) female wistar rats weighing 180-250 g were used for the study. Ethical clearance was obtained from the Ahmadu Bello University Animal Ethical Committee. Experimental animals in the study were treated in accordance with the National Protection Laws of Animal Welfare. The rats were divided into four groups of 20 rats each comprising 10 treated and 10 control rats. The treated groups received COC (DUOFEM) 0.6 mg/kg body weight intragastically for 36, 48, 60 and 72 days in 5-day cycles (4-day treatment with a 1-day break).An enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of Protein C and S Antigen in citrated rat plasma. Antithrombin (AT) was determined by Chromogenic Assays. Prothrombin time (PT), Activated partial thromboplastin time

Table 1: The effects of combined oral contraceptives on some anticoagulants in female wistar rats

GROUPS	AT (u/ml)	PC (u/ml)	PS (u/ml)
A (36 Days)	0.55±0.03	0.64±0.05	0.68±0.06
Control	0.62±0.06	0.75±0.08	0.82±0.09
P-value	0.022	0.012	0.001
B (48 Days)	0.48±0.05	0.52±0.06	0.67±0.04
Control	0.68±0.04	0.72±0.04	0.74±0.06
P-value	0.011	0.001	0.005
C (60 Days)	0.46±0.07	0.56±0.03	0.58±0.03
P-value	0.68±0.06	0.74±0.08	0.78±0.05
Control	0.001	0.008	0.001
D (72 Days)	0.43±0.06	0.48±0.05	0.58±0.07
Control	0.72±0.08	0.76±0.07	0.81±0.08
P-value	0.005	0.001	0.002

(APTT) were performed using Sysmex CA-6000 Coagulation Analyzer.

Statistical Analysis

The result obtained from this study was analyzed using SPSS version 20 for windows.Analysis of Variance (ANOVA) was used to compare means, and values werecompared at $P < 0.05$. Post Hoc multiple comparisons for significant differencesbetween groups were established by Turkey's HSD. All the data are expressed as Mean± Standard Error of Mean (SEM).

RESULTS

The result shows the serum level of AT III, PC and PS were statistically decreased. The serum level of AT III, PC and PS decreased with increase in the number of days the rats were exposed to the drugs ($P<0.05$)(Table 1).Prothrombin Time(PT) was significantly increased in all treated groups compared to the controls ($P<0.001$)(Figure 1). Activated Prothromboplastin Time (APTT) was not significantly changedin all the treated groups compared to the controls ($P<0.114$)(Figure 2).Fibrinogenwas significantly decreased in all the treated groups compared to the controls ($P<0.001$) (Figure 3).

DISCUSSION

Development of hormonal contraceptive marked a revolutionary step in social change that has improved the lives of women and families worldwide. Oral contraceptives are currently among the most commonly used drugs in developed countries. The most frequently used agents are the combination of drugs containing both oestrogen and progesterone.In this study the effect of COC on haemostatic parameters in female wistar rats was investigated.There were decreased levels of AT, PC and PS activity in the serum of the rats exposed to COC ($P < 0.05$). There was increased reduction in the serum levels of AT, PC and PS with increase in the number of days of exposure to COC ($P < 0.05$). This finding is in agreement with that of Norris and Bonnar,[9]Rosing,[3] and Soare and Popac,[10] who observed lower value of protein C and S in women using oral contraceptives containing disogestrel and ethimylestradiol.

COC has been linked to an increased incidence of thrombovascular disease. A reduction in AT level in women taking COC has been reported in some studies. Long-term use of COC and of hormone replacement therapy (HRT) has been linked with increase blood coagulation with its increased risk of cardiovascular diseases.[11][12][13] A reduction in the serum of AT, PC and PS as observed in rats given oral contraceptive could account for the alteration in coagulation in those taking COCs.

Proteins C and S and antithrombin are components of the anticoagulant system. In haemostasis, the procoagulant system is in balance with the anticoagulant and fibrinolytic system.[14] A decrease in AT, PC and PS may cause thrombosis. This finding is in agreement with that of Soare and Popac[10] and John and Emmanuel[15] who observed lower values of PC in women using oral contraceptives. Decreased AT, PC and PS may be responsible for the changes observed in haemostasis.

There was decrease fibrinogen levels in all the COC treated groups compared to the control. This finding differs from that of Eliana and Koni.[16] and Peter*et al.*[17] who reported increase in

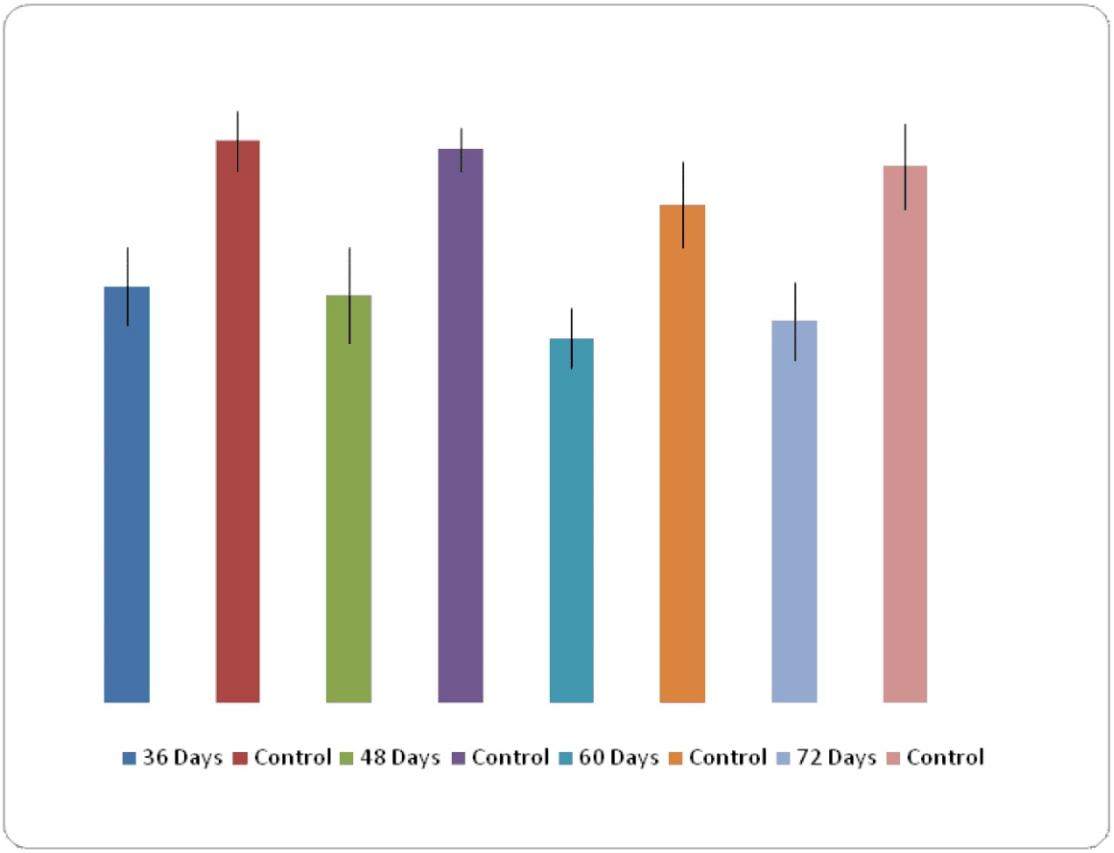

Fig 1: The effects of combined oral contraceptives on PT in female wistar rats

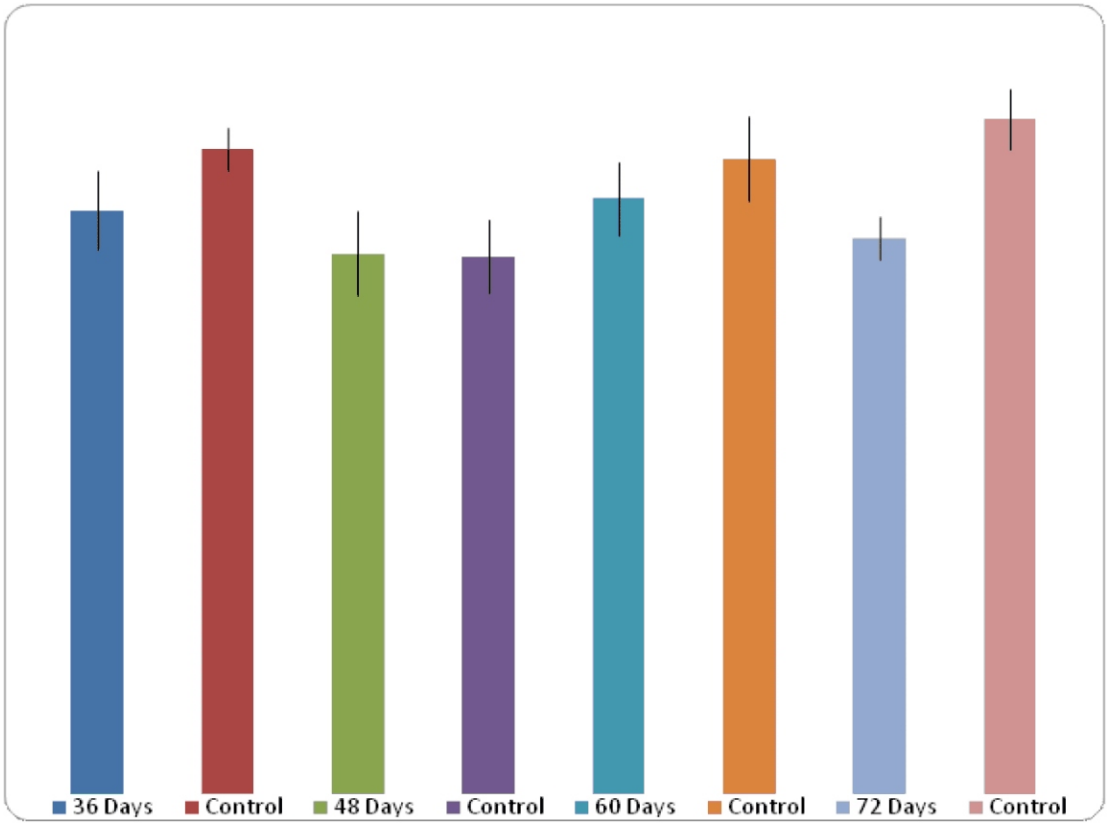

Fig 2: The effects of combined oral contraceptives on APTT female wistar rats

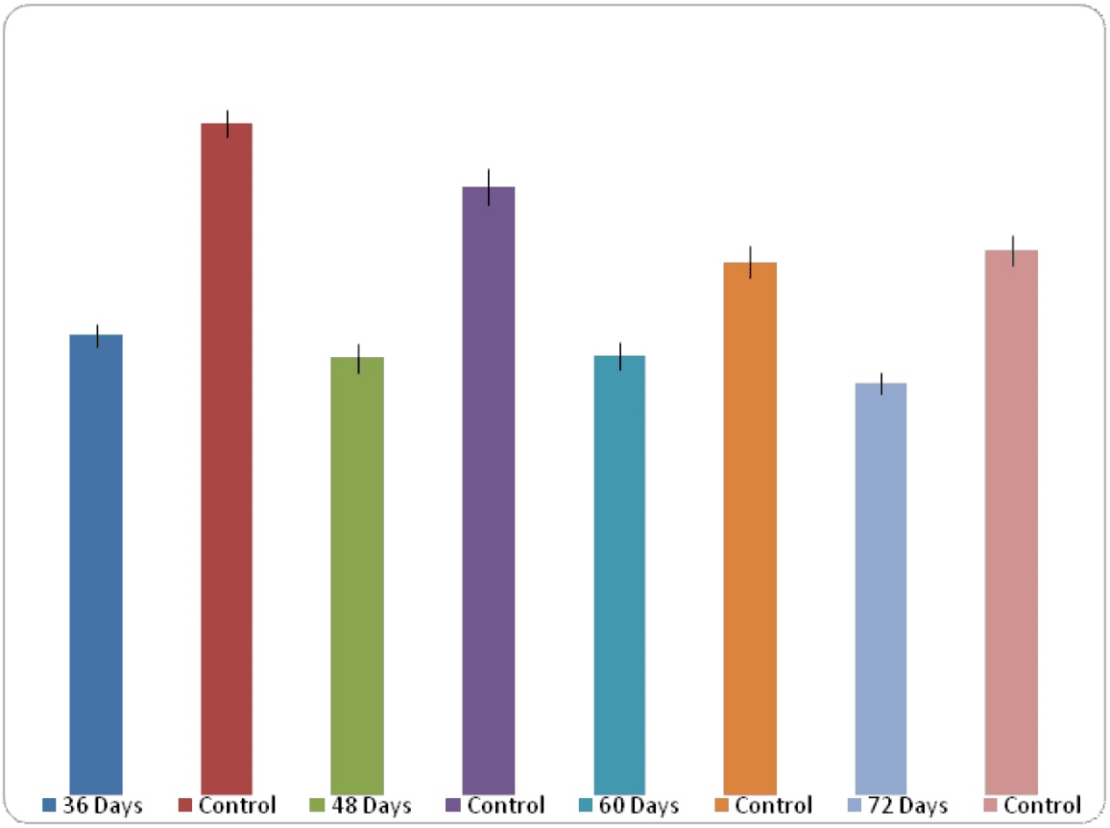

Fig 3: The effects of combined oral contraceptives on Fibrinogen in female Wistar rats

fibrinogen level in women taking COC. The finding is also contrary to that of Akhigbe*et al.*[18] who found no significant change in fibrinogen level in female wistar rats treated with COC.

The association between increase in plasma fibrinogen and thrombosis and the risk of myocardial infarction are well established. Higher level of fibrinogen raises the risk of stroke. Damage to the liver may be the cause of deficiency in fibrinogen level by a third generation COC (Duefem).This may reduce risk of thrombosis in users. Prothrombin Time (PT) significantly reduce in all the COC treated groups compare to the controls ($P<0.001$). There was no significant reduction in Activated Prothrombin Time (APTT). This finding agrees with that of Abdalla*et al.*[19] in Sudan found significant reduction in PT and APTT in women taking COC. Babatunde*et al.*[20] found that there was no significant change in the level of APTT in Nigerian women taking COC for three months. This is in agreement with the finding of this study.

Also Eliana and Koni[16] found reduced PT and APTT in Albanian women taking COC. Reduction in prothrombin time may be as a result of reduced serum level of fibrinogen. Estrogen is also said to increase coagulation factors leading to decreased prothrombin time. The finding of this study is also in agreement with that of Ahmed and Muna[21] and Nasir*et al.*[22] who reported a significant reduction in APTT and PT in women taking COC. The COC (DUOFEM) used in this study is likely to cause prothrombotic effect. This may lead to hypercoagulability and thrombosis.

CONCLUSION

COCs have evolved from one generation to another. The COC (DUOFEM) used in this study is a third generation COC. Much emphasis has been laid on the reduction of the concentration of estrogen and progesterone. Even at the lowest concentration of estrogen and progesterone in DUOFEM, the safety of COC is yet to be achieved. COC users should be monitored for haemostatic parameters.

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