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Full Length Research Paper

Effects of vitamin E and folic acid on some antioxidant enzymes activities of female Wistar rats administered combined oral contraceptives

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In this study, the effects of vitamin E and folic acid on the superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) production and glutathione-S-transferase (GST) activities in female Wistar rats treated with combined oral contraceptives (COC) containing ethinyl estradiol in combination with levonorgestrel were determined. Twenty female rats were divided into four groups: Group A (control) received distilled water; Group B received combined oral contraceptives (COC) for 15 days with a dosage of 0.667 mg/kg body weight/day; Group C received combined oral contraceptive and vitamin E (0.667 mg/kg body weight COC + 15 mg/kg body weight of vitamin E/day) for 15 days and Group D received combined oral contraceptives and folic acid (0.667 mg/kg body weight of COC + 1 mg/kg body weight/day) for 15 days. Administration of vitamin E and folic acid caused significant decrease ($P < 0.05$) in superoxide dismutase levels by 90 and 69% respectively. In catalase, administration of vitamin E significantly decrease ($p < 0.05$) catalase level by 47% while administration of folic acid has no significant difference ($p > 0.05$) in catalase level. In addition, administration of vitamin E and folic acid caused significant decrease ($P < 0.05$) in malondialdehyde (MDA) concentration by 21 and 11% respectively. Administration of vitamin E and folic acid caused significant decrease ($P < 0.05$) in glutathione-S-transferase by 39 and 23%, respectively.

Key words: Combined oral contraceptives, vitamin E, folic acid, antioxidant enzymes.

INTRODUCTION

Oral contraceptive, or birth control pills, is primarily used to prevent pregnancy and to treat menstrual irregularities and endometriosis (Gaspard et al., 2004). When taken as directed, they prevent ovulation. The female hormones estrogen and progestin are the agents in oral contraceptives that prevent ovulation. The use of oral contraceptive agents has been reported to be associated with a number of metabolic changes. Biochemical changes suggestive of altered nutritional status with regard to several vitamins such as folic acid (Prasad et al., 1975), vitamin E (Aftergood et al., 1974), ascorbic acid (Princemail et al., 2007) and vitamin A (Gaafar et al., 1973) have been reported among women who use contraceptive agents. Vitamin E (-tocopherol) is traditionally recognised as the most biological antioxidant in human (vitamin E Fact Sheet). Moreover, hormonal contraceptives are medicines which contain artificially made hormones which regulate women's menstrual cycles (Ross-Flanigan, 1999). Female hormones, such as estradiol have been reported to have strong inhibitory effects on lipid peroxidation and *in vivo* and *in vitro* antioxidant effects (Subbiah et al., 1993). In living system and aerobic organism, a complex antioxidant mechanism have been evolved to protect against uncontrolled free radical damage (Murray, 2002). However, there are

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evidences from studies in rats that the administration of contraceptive steroids significantly lowers plasma tocopherol levels and increases dietary requirements for vitamin E (Aftergood et al., 1974). In several cases, women taking oral contraceptive equally developed folic acid deficiency (Hathcock, 1997). The aim of this study was to assess the effects of vitamin E and folic acid supplementations on female Wistar rats administered combined oral contraceptive and to evaluate antioxidant enzymes such as lipid peroxidation, superoxide dismutase, catalase and glutathione-S-transferase.

MATERIALS AND METHODS

Drugs

The contraceptives drugs used were purchased from a family planning clinic in Abeokuta. Combined oral contraceptive pills (COC) DUOFEM® tablets, which contained ethinyl estradiol and levonorgestrel were manufactured by Pfizer, Belgium. Vitamin E was manufactured by Korea United Pharmaceutical Inc. 40410, South Korea and folic acid was manufactured by Emzor Pharmaceutical Industries Ltd. Lagos, Nigeria.

Clinical recommended daily allowance (RDA) dosage of each drug was prepared as concentration adjusted to mg/kg body weight of animals in the groups. Other reagents were of analytical grade and of the purest quality available.

Animals

Inbred of 5 – 6 week old female Wistar albino rats that weighed between 200 g and 240 g were purchased from the Animal House of College of Veterinary Medicine (COLVET), University of Agriculture, Abeokuta, Ogun State, Nigeria. The animals were kept in well-ventilated cages at room temperature (28 – 30°C) and under controlled light cycles (12 h light: dark) with humidity (54 - 57%). They were maintained on normal laboratory chow (Ola Feed Mills, Asero, Abeokuta, Nigeria) and water was by *ad libitum*. All experiments were conducted without anaesthesia and the protocol conformed to the guidelines of the National Institutes of Health (NIH publication no. 85 – 23, 1985) for laboratory animal care and use.

Experimental design and administration of drugs

20 female albino rats (Wistar strain) were randomly distributed into four groups of five animals each and were allowed free access to feed and water for a period of 2 weeks for acclimatization before the commencement of the experiment. The first group served as the control and received only distilled water. The second group received combined oral contraceptive (COC) only. The third group received combined oral contraceptive with vitamin E and the fourth group was administered combined oral contraceptive with folic acids. The contraceptive was administered orally for 15 consecutive days. The drug dosages for the rats were 0.667 mg/kg body weight for combined oral contraceptive, 15 mg/kg body weight for vitamin E and 1 mg/kg body weight for folic acid.

Sample collection

Preparation of serum

Rats were sacrificed 24 h after the last dose of drugs and an overnight fast. Blood was collected from the inferior *vena cava* of the heart of the animals into plain centrifuge tubes and was allowed to stand for 1 h. Serum was prepared by centrifugation at 3000 ×g for 15 min in a centrifuge. The clear supernatant was used for the estimation of serum enzymes. All procedures were carried out at temperatures 0 – 4°C.

Assay methods

Superoxide dismutase, catalase and glutathione S-transferase determination

Superoxide dismutase (SOD) activity was measured by the nitroblue tetrazolium reduction method of McCord and Fridovich (1969). Glutathione S-transferase (GST) activity was determined by the method of Habig et al. (1974); the method is based on the rate of conjugate formation between glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene. Catalase (CAT) activity was assayed by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi (1974).

Lipid peroxidation determination

The extent of lipid peroxidation (LPO) was estimated by the method of Buege and Aust (1978). The method involved the reaction between malondialdehyde (MDA), product of LPO and thiobarbituric acid to form a pink precipitate, which was read at 535 nm spectrophotometrically.

0.4 ml of reaction mixture already quenched with 0.5 ml of 30% TCA was added to 1.6 ml of Tri-KCl. Addition of TBA and incubation for 45 min at 80°C produced pink coloured reaction mixtures which were centrifuged at 14000 g for 15 min. The absorbance of the clear pink supernatant was then read at 535 nm.

Statistical analysis

All values were expressed as the mean ± S.D of five animals per group. Data were analysed using one-way ANOVA test followed by the *post-hoc* Duncan's multiple range test for analysis of biochemical data using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). Values were considered statistically significant at $P < 0.05$.

RESULTS

Effect of combined oral contraceptives, vitamin E and folic acid supplements on lipid peroxidation in female Wistar rats

The effect of vitamin E and folic acid on lipid peroxidation in combined oral contraceptive treated female rats was assessed. The combined oral contraceptives dose (0.667 mg/kg body weight) produced no significant difference ($p > 0.05$) in MDA level compared to the control while vitamin E (15 mg/kg body weight) produced significant reduction ($p < 0.05$) of MDA level by 21% compared to group B (combined oral contraceptive dose only). The folic acid (1 mg/kg body weight) equally produced significant reduction ($p < 0.05$) of MDA level by 11% compared to group B (combined oral contraceptive dose only) as shown in Figure 1.

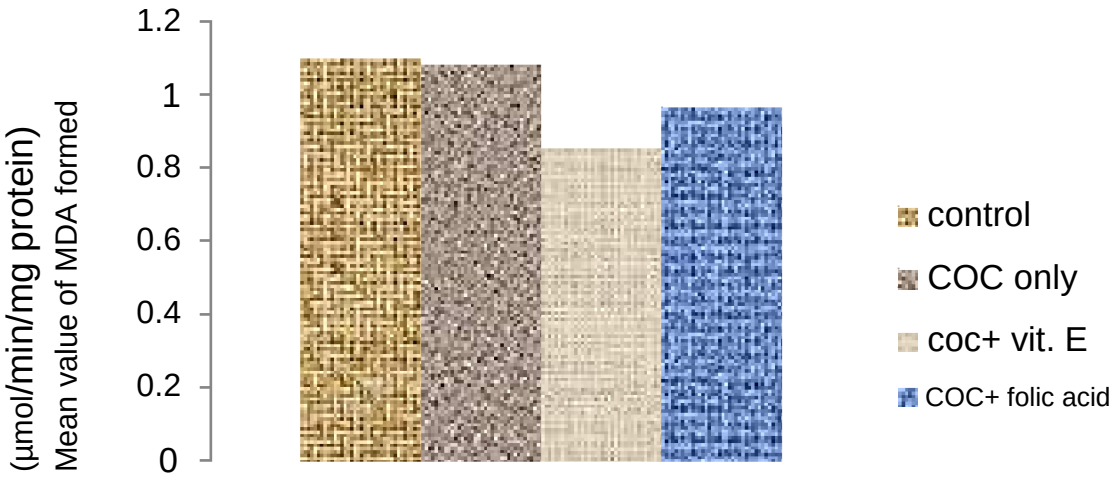

Figure 1. Effect of combined oral contraceptives, vitamin E and folic acid supplements on lipid peroxidation in female Wistar rats.

Effect of combined oral contraceptives, vitamin E and folic acid supplements on superoxide dismutase, glutathione-S-trasferase and catalase enzymes activities in female Wistar rats

The effect of vitamin E and folic acid supplements on superoxide dismutase (SOD), glutathione-S-transferase (GST) and catalase activities in combined oral contraceptives treated female rats were assessed. The combined oral contraceptive dose (0.667 mg/kg body weight) produced significant elevation ($p < 0.05$) of SOD, GST and catalase activities by 87, 25 and 41% respectively compared to the control while vitamin E and folic acid produced significant reduction ($p < 0.05$) in superoxide dismutase (SOD) by 90 and 69% respectively when compared to group B (combined oral contraceptive dose only). Administration of vitamin E and folic acid, significantly decrease ($p < 0.05$) glutathione-S-transferase levels by 39 and 23% respectively when compared to group B. However, administration of vitamin E and folic acid, significantly decrease ($p < 0.05$) catalase level by 47% but there was no significant decrease ($p > 0.05$) in catalase level by folic acid administration when compared to group B as shown in Figures 2, 3 and 4.

DISCUSSION

In this study, one major observation is that combined oral contraceptive pills (COC) containing ethinyl estradiol and levonorgestrel had significant effect ($p< 0.05$) on all the antioxidant markers but in exception to lipid peroxidation and this is in agreement with the observation of Subbiah et al. (1993) and Kose et al. (1993). They both observed that estradiol have strong inhibitory effects on lipid peroxidation. However, the effects of vitamin E and folic acid supplementation on the antioxidant markers after the treatment of the female rats with combined oral contraceptive are remarkable. Based on the MDA assay, vitamin E and folic acid reduced the effects of the COC significantly ($p < 0.05$) and this is in accordance to the findings that vitamin E is a powerful antioxidant (Obikoya, 2008).

Glutathione-S-transferase (GST) is a family of multifunctional isozymes found in all eukaryotes, catalysing both glutathione dependent conjugation and reduction reactions (Rujurkar et al., 2003; Adaramoye et al., 2008). It can also act as an antioxidant enzyme (Adaramoye et al., 2006). In this study, GST activity was elevated in rats treated with combined oral contraceptives; this also suggested that COC may have elevated oxidative stress in the female rats. Consequently, the administration of vitamin E and folic acid significantly altered ($p < 0.05$) the effect of the COC which equally collaborated with the antioxidant ability of vitamin E.

In the superoxide dismutase (SOD) and catalase results, an equal trend was observed suggesting the corrective ability of vitamin E and folic acid on oxidative stress that may arise following administration of combined oral contraceptives.

Conclusion

The findings suggested that vitamin E and folic acid reduced significantly the effect of the combined oral contraceptives on female treated rats which is in agreement with evidence from previous studies in rats that the administration of contraceptive steroids significantly lowers plasma tocopherol levels and increases dietary requirements for vitamin E. Therefore, it will be suggested that women on combined oral contraceptives should be on vitamin E and folic acid

Table 1. Effect of combined oral contraceptives, vitamin E and folic acid on lipid peroxidation, superoxide dismutase (SOD), catalase and glutathione-S- transferase (GST) activities.

Groups of rats fed with combined oral contraceptive (COC)	Concentration of MDA x10^{-2} (µmol/min/mg protein)	Superoxide dismutase activity x10^{-2} (U/mg protein)	Catalase activity x10^{-2} (µmol/min/mg protein)	Glutathione S -transferase activity x10^{-2} (µmol/min/mg protein)
A (Control)	1.105±0.27	0.372±0.06	0.375±0.14	0.884±0.19
B (Combined oral contraceptive)only	1.084±0.13*	2.896±1.41*	0.635±0.06*	1.183±0.81*
C (COC + Vitamin E)	0.854±0.07*	0.289±0.07*	0.335±0.02*	0.715±0.49*
D (COC+ Folic acid)	0.967±0.22	0.897±0.10*	0.594±0.07	0.910±1.07*

COC, Combined oral contraceptive; MDA, malondialdehyde. Values are means ± S.D of five animals per group. *Significantly different from control (P < 0.05).

Figure 2. Effect of combined oral contraceptives, vitamin E and folic acid supplements on superoxide dismutase enzyme in female Wistar rats.

Figure 3. Effect of combined oral contraceptives, vitamin E and folic acid supplements on catalase activity in female Wistar rats.

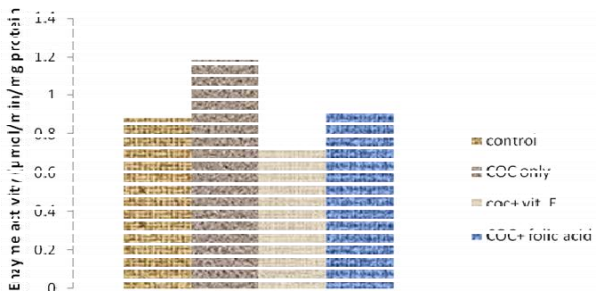

Figure 4. Effect of combined oral contraceptives, vitamin E and folic acid supplements on glutathione-S-transferase activity in female Wistar rats.

supplementation to reduce any oxidative stress that may arise from its use. Further researches on lipids profile components and enzymes activities in human subjects are in progress.

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