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Biochemical Potential of Vitamin C as the Free Radical Scavenger and Increase Reduced Glutathione (GSH) in Brain of Female Rat Administered Combined Oral Contraceptives.

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Abstract
Background
Several millions of women in reproductive age all over the world make use of combined oral contraceptive to prevent unwanted pregnancy and treat menstrual abnormalities.Most combined oral contraceptives has been shown to induce oxidative stress in tissues such as brain. Since vitamin c has been shown with high antioxidant probabilities in many studies. The present study was undertaken to assess the potential of vitamin c as the free radical scavenger and increase reduced glutathione (GSH) in brain of female rat administered combined oral contraceptives.
Method
The experiment was performed in 24 female Wistar rats weighing 180g - 250 g. The animals were divided into 4 groups: Group 1(control), group 2(Vitamin C only) (150mg/kg body weight). group 3(Combined Oral Contraceptive only) (0.667mg/kg body weight)and group 4(Combined Oral Contraceptive and vitamin C only) . After completing the experiments, the brains of all animals were collected and homogenized for further biochemical determination of antioxidant enzyme activities and vitamin C level.Data were analyzed using two-way ANOVA test followed by the Bonferroni's multiple comparison test for analysis of biochemical data using SPSS version 10.0 .
Results
It was observed that group 3 treated with combined oral contraceptives only showed decreased GSH activity accompanying with oxidative stress, indicating by, the increased MDA activity and The beneficial effects of vitamin C treatment were shown in group 4 treated with combined oral contraceptives and vitamin C only accompanying with reduced oxidative stress. The effects are likely in dose-dependent manner.
Conclusion
This study indicates that vitamin c is effective in decreasing oxidative stress in the brain and help recovery of damages in cognitive function caused by combined oral contraceptives
Keywords: Combined Oral Contraceptive,Vitamin C,Brain and antioxidant enzymes

INTRODUCTION

Women in reproductive age all over the world have made use of oral contraceptive agents to prevent unwanted pregnancy and treat menstrual abnormalities. The agent present in the oral contraceptive are the oetrogen and progestin which prevents pregnancy and to treat menstrual irregularitiesand endometriosis (Gaspard et al., 2004).Evidence that Combined Oral Contraceptive produces side effects such as oxidative damage in tissues like brain.The brain exhibits distinct variations in cellular as well as regional distribution of antioxidant biochemical defenses (Verma and Srivastava, 2001).Combined Oral Contraceptive also results in biochemical changes such as altered nutritional status with regard to several vitamins such as vitamin C (Princemail et al., 2007).Vitamin C is the most abundant water-soluble vitamin found in many plants and fruits and is also an essential nutrient for humans and a few other animals(Hancock ,2005).Neural cells and/or brain regions are likely differentially respond to changes in metabolic rates associated with the generation of reactive oxygen species (Hussain et al., 1995) .This present study was undertaken to assess the potential of vitamin c as the free radical scavenger and increasereduced glutathione (GSH) in brain of female rat administered combined oral contraceptives.

MATERIALS AND METHODS

Drugs

Combined oral contraceptive pills (COC) DUOFEM® tablets, which contained ethinyl estradiol and levonorgestrel were manufactured by Pfizer, Belgium was used. Vitamin C was manufactured by Agary Pharmaceutical Limited ,Amuwo-odofin,Lagos,Nigeria and ferrous fumarate was manufactured by Pfizer, Belgium.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
24 female rats that weighed between 180 g and 240 g were purchased from the animal house of College of Medicine, University of Lagos,Lagos Nigeria.

EXPERIMENTAL DESIGN AND ADMINISTRATION OF DRUGS.
24 female rats were randomly distributed into four groups of six animals each and were allowed free access to feed and water for a period of two weeks for acclimatization before the commencement of the experiment. Group 1(control), group 2(Vitamin C only) (150mg/kg body weight). group 3(Combined Oral Contraceptive only) (0.667mg/kg body weight)and group 4(Combined Oral Contraceptive and vitamin C only).The contraceptive was administered orally for 21 consecutive days.

Biochemical Tests
At day 21, animals were decapitated under pentobarbital sodium anaesthesia and their brains quickly removed,weighed, cleaned with ice-cold saline and stored at -80 oC.

Tissue preparation
Brain tissue samples were thawed and homogenized with 10 times (w/v) ice-cold 0.1 mol/l phosphate buffer (pH 7.4). The homogenates were centrifuged at 15,000 g for 60 min and the supernatant was then used to determine lipid peroxidation and glutathione.

Biochemical Analysis Performed Included
Antioxidant Enzymes:
1) **Determination of superoxide dismutase activity:** Brain SOD activity was determined according to the method of Sun et al.1988. The principle of the method is based on the inhibition of nitroblue-tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate and the results were expressed as U/mg protein.
2) **Determination of catalase activity:** Brain catalase activity was determined according to Aebi's method, 1974). The principle of the assay is based on the determination of the H2O2 decomposition rate at 240 nm. Results were expressed as U/mg protein. S
3) **Measurement of Reduced Glutathione:** Glutathione was measured according to the method of Ellman,1959. An equal quantity of homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate separate the proteins. To 0.01 ml of this supernatant, 2 ml phosphate buffer (pH 7.4), 0.5 ml 5û5-dithiobis (2-nitrobenzoic acid) and 0.4 ml distilled water were added. The mixture was vortexed and the absorbance read at 412 nm within 5 min. The concentration of reduced glutathione was expressed as µmol/min/mg tissue.
4) **Determination of malondialdehyde level**: The brain MDA level was determined by a method (Okhawa et al., 1979) based on the reaction with thiobarbituric acid (TBA) at 90–100 oC. After cooling, the absorbance was read at 532 nm. Results were expressed as nmol/g protein.
5)**Vitamin C Assay :** Vitamin C was estimated by the colorimetric method (Omaye et. al., 1979) 0.5 ml of tissue homogenate , 0.5 ml of distilled water and 1.0 ml of 10% TCA were added, mixed thoroughly and centrifuged for 20 minutes. 1.0 ml of the supernatant and 0.2 ml of (DTC reagent) 2,4 dinitro phenylhydrazine–thiourea–copper sulphate reagent was added and incubated at 37°C for 3 hours. Then 1.5 ml of 65% sulphuric acid was added, mixed well and allowed to stand at room temperature for another 30 minutes. The color developed was read at 520 nm. Graded amount of standards were also treated similarly. Vitamin C level was expressed as µg/mgof wet brain.

Statistical analysis
Data were analysed using two-way ANOVA test followed by the Bonferroni's multiple comparison test for analysis of biochemical data using SPSS version 10.0 . Values were considered statistically significant at $P < 0.05$

RESULTS
Biochemical potential of vitamin c as the free radical scavenger and increase reduced glutathione (GSH) in brain of female rat administered combined oral contraceptives.on malondialdehyde (MDA),superoxide dismutase (sod) catalase (CAT), reduced glutathione(GSH) and vitamin c level in the brain of female rats administered combined oral contraceptive in the brain of female rats.

	Group 1 CONTROL	**Group 2** VITAMIN C ONLY	**Group 3** COC ONLY	**Group 4** COC +VIT C
Brain biochemical Marker				
MDA (µmol/mg protein x 10^{-2})	0.68 ±0.05	0.64±0.08	1.06±0.33*	0.86±0.08*
GSH (µmol/min/mg protein x 10^{-2})	0.95±0.07	1.03±0.03	0.75±0.14*	0.84±0.01
SOD U/mg protein.	0.42±0.04	0.32±0.02*	0.65±0.03*	0.56±0.01
CAT U/mg protein.	0.38±0.02	0.34±0.02*	0.59±0.01*	0.41±0.05
VITAMIN C (µg/mg protein)	383.0±98.08	450.3±69.79*	326.5 ±63.91*	372.5±54.53*

Values are means ± Standard Error of Mean of six animals per group. *Significantly different from control (P < 0.05)

RESULTS

Biochemical potential of vitamin C on malonyaldehyde in brain of female rats administered combined oral contraceptive was assessed. The combined oral contraceptives dose (0.667mg/kg body weight) produced significant increase ($p > 0.05$) in MDA level compared to the control while vitamin C (150 mg/kg body weight) produced significant reduction ($p < 0.05$) of MDA level in group 4 (combined oral contraceptive and vitamin c only) compared to group Superoxide dismutase (SOD)and Catalase(CAT) activities in brain of female rats administered combined oral contraceptive was assessed.The combined oral contraceptive dose (0.667 mg/kg body weight) produced significant elevation ($p < 0.05$) of SOD and CAT activities compared to the control while vitamin C produced significant reduction ($p < 0.05$) in superoxide dismutase (SOD) compared to group 3 (combined oral contraceptive dose only). Administration of vitamin C significantly increase ($p < 0.05$) the level of reduced gluthathione compared to group 3.which the level of reduced gluthathione is reduced.However, administration of vitamin C significantly decrease ($p < 0.05$) catalase level but there was no sigificant decrease in catalase ($p > 0.05$) compared to group 3 as shown in table above.sThe vitamin C level was also assessed in the brain.The level of vitamin C also decreased significantly in group 3(COC only) compared to the control.

DISCUSSION

The biochemical analyses assessed showed a pattern of altered redox homeostasis in the brain as indicated by a lower vitamin C level and higher level of antioxidant enzymes in the brain of group 3 rat(COC only) compared to the group 1(CONTROL) .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 biomarkers including the lipid peroxidation and this is in agreement with the observation of Tang et al. (1996) and Tranquilli et al. (1995). They both observed that estradiol reduces lipid peroxidation in plasma and platelet membranes in post menopausal woman. However, biochemical studies of vitamin C on the antioxidant markers after the treatment of the female rats with combined oral contraceptive are noticeceable. According to the MDA assay, vitamin C reduced the effects of the COC significantly ($p < 0.05$) and this is in accordance to the findings that vitamin C In the brain which plays a pivotal role in maintaining redox homoeostasis (Tveden-Ny ET, AL,borg, 2009).
GSH plays a vitally important role in cellular function; in fact the maintenance of GSH

Homeostasis is essential for the organism to perform its many functions.In this study, GSH activity was elevated in rats treated with combined oral contraceptives; this also suggested that COC may have elevated oxidative stress in the brain of female rats.Consequently, the administration of vitamin C significantly altered ($p < 0.05$) the effect of the COC which is in accordance with the antioxidant ability of vitamin C.

In the superoxide dismutase (SOD) and catalaseresults, an equal trend was observed suggesting the ameliorative ability of vitamin C on oxidative stress that may arise following administration of combined oral contraceptives. Ascorbate enters the extracellular fluidby diffusion from the cerebrospinal fluid or through capillariesin the blood-brain barrier, possibly through facilitated GLUT transport (Agus et., al., 1997). Relatively high concentrations of vitamin C in the brain during reduced plasma and tissue vitamin C concentrations indicate that the brain is favored during deficiency, which indicates an essential role of vitamin C in the brain (Kuo et., al., 1979). Vitamin C level lowered in of the brain female rats exhibits disabilities associated with neuronal dysfunction such as depression with increased oxidative stress (Harrison et al, 2008). The present findings show

that vitamin C treatment attenuated lipid peroxidation in the rat brain. This was proved by the decreased MDA level, accompanied by increased GSH and vitamin C content and enhanced activities of CAT, SOD enzymes. These results could be attributed to the potential antioxidant effect of vitamin C

Conclusion

From the above observation it can be concluded that, combined oral contraceptives has negative effect on vitamin C level in brain tissue of rats. But when Vitamin C was treated along with combined oral contraceptives the levels of Vitamin C also improved. Therefore, it will be suggested that women on combined oral contraceptives should be on vitamin C.

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