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Effect of Two Combined Oral Contraceptives On Selected Oxidative Stress Parameters and Lipid Profile In Female Rats

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(Microgynon and Nordiol)

(GSH, MDA, CAT and GST)

Sprague-Dawely

(P<0.05) (GSH)

(MDA, CAT and GST).

(P<0.01)

(GSH, CAT, MDA and GST).

(P<0.05) (P<0.01).

Abstract:

The present study was undertaken to assess the effect of two combined oral contraceptives: Microgynon (ethinyl estradiol (EE2) 0.004906mg/kg and levonorgestrel (LNG) 0.024533mg/kg) and Nordiol (EE2 0.008177mg/kg and LNG 0.040889mg/kg) on selected oxidative stress parameters: reduced glutathione (GSH), lipid peroxidation byproduct malondialdehyde (MDA), catalase activity (CAT) and glutathione S-transferase enzyme activity (GST) and their effect on serum lipid profile(total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in female rats which were maintained on high cholesterol diet. Thirty female Sprague–Dawely rats were enrolled in this study, 6 rats were maintained on normal standard chow diet and considered as control group, the other 24 rats received high cholesterol diet for 14 weeks, then blood samples were taken randomly from 6 rats which underwent laparotomy, and experimental parameters were measured. The remaining 18 rats were randomly separated into 3 groups, six rats in each group; one group continued on high cholesterol diet and distilled water for 6 weeks and served as self control group. The other 2 groups continued on high cholesterol diet and one of the oral contraceptives mentioned above for 6 weeks. Then, the selected parameters were measured at the end of the 6 weeks of treatment. **Results**: Microgynon treatment decreased serum GSH significantly while did not significantly affect MDA level, CAT and GST activity. Microgynon treatment decreased serum high density lipoprotein (HDL) level significantly, while did not significantly affect serum TC, TG, LDL and VLDL. Nordiol treatment did not significantly affect serum level of GSH, MDA, CAT and GST. In Nordiol treatment serum TG and VLDL levels increased significantly, serum HDL level decreased significantly, other parameters of lipid profile did not significantly change.

Introduction: Oxidative stress is defined as an imbalance between prooxidants (free radical species) and the body's scavenging ability (antioxidants).(1) More severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis.(2) A particularly destructive aspect of oxidative stress is the production of reactive oxygen species(ROS)(3), which are chemical species that have a single unpaired electron in an outer orbit. They are highly reactive chemical radicals that are generated as products of oxygen degradation.(4) They are molecules like hydrogen peroxide, ions like the hypochlorite ion, radicals like the hydroxyl radical, both ion and radical like superoxide anion.(5) Oxidative stress plays an important role in the DNA damage, Lipid peroxidation(6) and in the pathogenesis of atherosclerosis, ROS are also involved in a diversity of biological phenomena including radiation damage, carcinogenesis, ischemia–reperfusion injury, diabetes mellitus, neurodegenerative diseases(7), cardiovascular disease, the aging process(8-10), as well as Alzheimer disease [11, 12] cataract and emphysema.(13) In addition, elevated ROS in breast cancer cells has been proposed as a condition promoting metastasis.(14) Hyperlipidemia means that high levels of fats (or lipids) are in the blood.(15) Hypercholesterolemia is an important risk factor for the development and progression of atherosclerosis and premature coronary heart disease, the pathogenesis is multifactorial and includes vascular inflammation and increased generation of vascular superoxide, hydroxyl radicals, and hydrogen peroxide(16,17). Oral contraceptives (OCs) are medicines taken by mouth to prevent pregnancy. They contain

synthetic forms of two hormones produced naturally in the body, these hormones are estrogen and progestin, regulate the female menstrual cycle. Some types of oral contraceptives use only progestational hormones, but most use a combination of estrogen and progestin.(18) Combined oral contraceptives (COC) prevent pregnancy primarily by inhibiting ovulation through the combined actions of progestin and estrogen. The dominant component is progestin, which inhibits ovulation by suppressing the cyclical release of luteinizing hormone (LH) from the anterior pituitary gland. Progestins also create a thick cervical mucus that slows sperm transport and inhibits capacitation (the activation of enzymes that permits the sperm to penetrate the ovum). Estrogen in COCs contributes to ovulation inhibition by suppressing the release of follicle-stimulating hormone (FSH) and LH. Estrogen also accelerates ovum transport, which decreases fertilization time. Finally, estrogen alters secretions within the uterus to produce areas of edema and dense cellularity, making implantation less likely.(19,20). The most common side effects are nausea, weight gain, breast tenderness, headache(21) discomfort, swelling, depression or mood disturbances, decreased sexual desire or response, hypertension and acne. Rare but serious potential effects include cardiovascular diseases, such as stroke, and an increased risk for breast cancer, liver tumors, and gallbladder disease. Hormonal contraceptive use should be avoided in women at risk for blood clots, by heavy smokers, and in women with breast or other cancers.(22) Estrogens are known to be powerful antioxidants independently of their binding to the estrogen receptors and the hormonal functions.(23,24) **Laszlo Prokai *et al.,*** and **Laszlo Prokai *et al.,*** found that this activity is associated with the presence of the phenolic A-ring in the structure of these molecules, thus rendering them members of the class of compounds called phenolic antioxidants.(24,25) Progestins opposed the antioxidant effect of estrogen, with the strongest antiestrogenic effect seen with the synthetic progestins, levonorgestrel and medroxyprogesterone acetate.(26) Most combined contraceptive steroids currently used contain EE_2, so that any differences in their metabolic effects depend on the dose and type of progestin component.(27) Estrogen is widely regarded to have beneficial effects on lipid profile, these effects include a reduction in LDL, an increase in HDL, but it increases plasma triglyceride levels.(28,29) On the other hand, **Knopp RH.** had found that progestins appear to slow the stimulatory effect of estrogens on lipoprotein transport in the bloodstream.(28)

The aim of this study was to clarify the effect of COCs (high and low dose) on selected serum oxidative stress parameters: GSH, MDA and two antioxidant enzymes: CAT and GST, and their effect on lipid profile in female rat.

Materials and Methods: Thirty female adult Sprague-Dawely rats were enrolled in this study. The animals were obtained from the Animal House in Kufa Medical College. Their weight was (170-250)g and age between 3-6 months. The rats were housed in Kufa Medical College Animal House in (43 x 27 x 15)cm cages (3 rats in each cage) and kept at 25 ˚C and 12 hours light-dark cycles with 12.00 AM being the mid dark period. Rats had free access to drinking water and libitum. After 2 weeks of adaptation 6 rats were maintained on normal standard chow diet and considered as control group from which the baseline value of experimental parameters was measured.

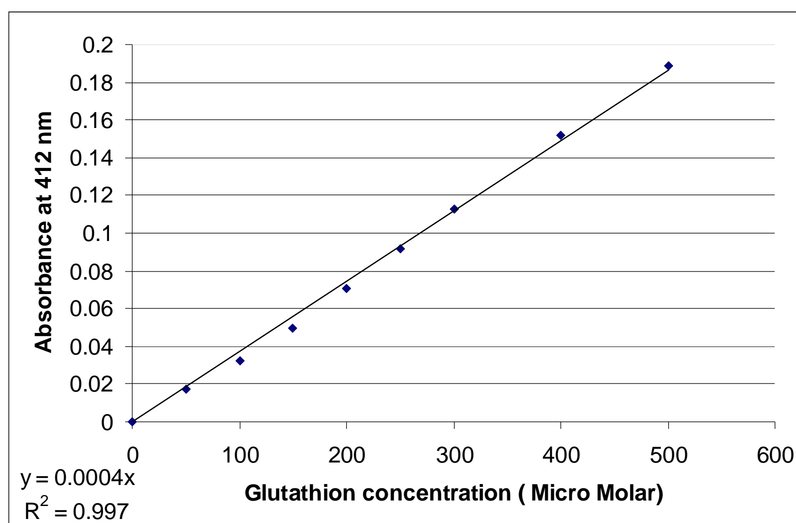
The other 24 rats received high cholesterol diet containing Cholesterol 3% by weight made by the addition of cholesterol powder (from Griffin Company, England) to the

chow plus cocconut oil 25% by weight,(30) for 14 weeks to develop hypercholesterolemia. After the 14 weeks blood samples were taken randomly from 6 rats which underwent laparotomy, and experimental parameters were measured. Then the remaining 18 rats were randomly separated into 3 groups, six rats in each group as follow:

Group1 continued on high cholesterol diet and distilled water orally by gastric tube for 6 weeks without drug treatment and served as self control. Group2 continued on high cholesterol diet and Microgynon (EE_2 0.004906 mg/kg + LNG 0.024533 mg/kg)by stomach tube for 6 weeks. Group3 continued on high cholesterol diet and Nordiol (EE_2 0.008177 mg/kg + LNG 0.040889 mg/kg) by stomach tube for 6 weeks. At the end of the 6 weeks, blood samples were taken from all rats which underwent laparotomy, and experimental parameters were measured.

Serum reduced Glutathione (GSH) assay: Serum (GSH) was estimated according to the method of Ellman[31], the used reagents were supplied by Biochemicals Co.Ltd. for EDTA and GSH, Sigma Co.Ltd for DTNB.

The assay mixture contained serum and DTNB 0.01 M (5,5-Dithiobis-2-nitrobenzoic acid), trichloroacetic acid (TCA 50%), Tris-EDTA buffer (0.2M) PH 8.9, $EDTANa_2$ (0.2M) and GSH standards for preparation of stock standard solution and standard calibration curve in micro M. The net results read at 412 nm by using (Shimadzu UV-1650P (UV-visible)) Spectrophotometer.



Serum lipid peroxidation product (MDA) assay: The level of serum MDA was determined by a modified procedure described by**(Guidet B. and Shah S.V.,1989)**[32]**.**All the chemicals were supplied by **Merck Co.ltd.** The assay mixture contained serum and 17.5% trichloroacetic acid (TCA), 70% TCA, 0.6% thiobarbituric acid (TBA) and (Shimadzu UV-1650P (UV-visible)) Spectrophotometer used to read the final sample at 532 nm. The concentration of MDA was expressed in molar.

Serum GST enzyme activity assay: Serum GST activity determined by Habig method.(33) The used reagents were supplied by Analar for CDNB, K_2HPO_4 and KH_2PO_4. Biochemicals Co. Ltd. for GSH. The assay mixture contained serum, reduced glutathione (GSH), 1-chloro,2,4-dinitro benzene(CDNB), Phosphate buffer (PH 6.25) and (Shimadzu UV-1650P(UV-visible)) Spectrophotometer used to read the final sample at 340 nm.

Serum catalase activity assay: Serum catalase activity determined by Hugo Aebi method[34].The used reagents were supplied by Analar for Na_2HPO_4 and KH_2PO_4 and H_2O_2. The assay mixture contained diluted serum, phosphate buffer, hydrogen peroxide (H_2O_2) 30 mM and (Shimadzu UV-1650P(UV-visible)) Spectrophotometer used to read the final sample at 240 nm.

Serum lipid profile assay: Total cholesterol, Triglyceride, High density lipoprotein was measured according to procedures supplied by Spinreact company, using (Shimadzu UV-1650P (UV-visible)) Spectrophotometer. Serum LDL measure according to the following equation:(35)

LDL= total cholesterol **-** HDL **-** VLDL

VLDL= TG/5.

Drugs used in the experiment: The dose of hormonal contraceptive pills in rodents per Kilogram of animal weight has been measured as 10 times the human dose [36].Considering the human dose is one tablet daily and the average weight of adult human female = 61.14 kg,(37) the dose is given once daily at the same time every day.

Microgynon: It was used in a dose of (EE_2 0.004906mg/kg + LNG 0.024533mg/kg) /day P.O, a tablet contains (EE_2 0.03mg and LNG 0.15mg) (MICROGYNON ED Fe (SCHERING)) was dissolved in distilled water and the dose was given to the rats according to the body weight once daily at the same time every day through stomach tube for 6 weeks.

Nordiol: It was used in a dose of (EE_2 0.008177mg /kg + LNG0.040889mg/kg) /day P.O,a tablet contains (EE_2 0.05mg and LNG 0.25mg)(NORDIOL (Wyeth-Pharma)) was dissolved in distilled water and the dose was given to the rats according to the body weight once daily at the same time every day by stomach tube.

Statistical analysis: The data expressed as mean ± SEM unless otherwise stated. Statistical analysis have been done by using independent t-test and LSD. Significant difference was set at =0.05.

Results:

Effect of 14 week Cholesterol Rich Diet on The Measured Parameters:

Serum TC level increased significantly (P<0.05), TG, LDL and VLDL levels increased significantly (P<0.01), HDL decreased significantly (P<0.01) after 14 weeks of cholesterol rich diet. While serum GSH, CAT, MDA, GST did not significantly change (P>0.05) **(Table 1).**

Table(1): Effects of 14 week cholesterol rich diet on selected oxidative stress parameters and lipid profile in female rats serum (No. = 6 rats in each group).

	Normal control Group	**Self control (after 14 weeks)**	**P. Value**
GSH (µM)	29±3.68	25.5±2.32	N.S
CAT (k/ml)	0.05±0.008	0.04±0.002	N.S
MDA (M)	$5.51*10^{-6}$±0.79	$7.6*10^{-6}$±1.44	N.S
GST (U/L)	21.61±4.35	16.4±1.05	N.S
TC (mg/dL)	45.15±1.62	53.8±3.68	P < 0.05
TG (mg/dL)	20.5±2.94	50.48±1.86	P < 0.01
HDL (mg/dL)	31.05±0.88	22.04±1.08	P < 0.01

LDL (mg/dL)	10.001±0.66	21.66±3.56	P < 0.01
VLDL(mg/dL)	4.1±0.58	10.09±0.37	P < 0.01

The values expressed as mean ± SEM

Effects of the treatments on oxidative stress parameters:

Oxidative stress parameters did not significantly change in self control and Nordiol groups. Microgynon treatment decreased serum GSH level significantly while did not significantly affect serum level of MDA, CAT and GST **(table 2,3).**

Table (2): Effects of 6 week treatment with Microgynon and Nordiol on GSH and CAT levels in female rats serum which were fed cholesterol rich diet. (No. = 6 rats in each group).

	Glutathione level (µM)			CAT activity (k/ml)		
	Before treatment	After treatment	P. Value	Before treatment	After treatment	P. Value
Self control group	25.5±2.32	24.2±4.45	N.S	0.04±0.002	0.04±0.003	N.S
Microgynon group	25.5±2.32	15.4±1.66	P <0.05	0.04±0.002	0.031±0.004	N.S
Nordiol Group	25.5±2.32	23.83±3.31	N.S	0.04±0.002	0.037±0.006	N.S

The values expressed as mean ± SEM

Table(3): Effects of 6 week treatment with Microgynon and Nordiol on serum MDA and GST levels in female rats serum which were fed cholesterol rich diet. (No. = 6 rats in each group).

	MDA level (M)			GST activity (U/L)		
	Before treatment	After treatment	P. Value	Before treatment	After treatment	P. Value
Self control group	7.60×10^{-6}±1.44	8.02×10^{-6}±0.83	N.S	16.4±1.05	15.93±1.48	N.S
Microgynon group	7.60×10^{-6}±1.44	8.59×10^{-6}±0.88	N.S	16.4±1.05	15.46±1.75	N.S
Nordiol Group	7.60×10^{-6}±1.44	8.9×10^{-6}±1.24	N.S	16.4±1.05	15.1±1.61	N.S

The values expressed as mean ± SEM

Effects of the treatments on lipid profile:

Lipid profile did not significantly change in self control group. Microgynon treatment decreased HDL level significantly (P<0.05) but had no significant effect on serum TC, TG, LDL and VLDL levels. Nordiol treatment decreased serum HDL level, increased TG and VLDL levels significantly (P<0.05), but had no significant effect on serum TC and LDL levels.**(Table 4,5,6)**

Table (4): Effects of 6 week treatment with Microgynon and Nordiol on TC and TG levels in female rats serum which were fed cholesterol rich diet. (No. = 6 rats in each group).

	TC level (mg/dL)			**TG level (mg/dL)**		
	Before treatment	**After treatment**	**P. Value**	**Before treatment**	**After treatment**	**P. Value**
Self control group	53.8±3.68	54.38±2.56	N.S	50.48±1.86	53.74±4.81	N.S
Microgynon group	53.8±3.68	49.97±1.71	N.S	50.48±1.86	65.44±6.84	N.S
Nordiol Group	53.8±3.68	47.18±1.39	N.S	50.48±1.86	70.45±4.86	P < 0.05

The values expressed as mean ± SEM

Table (5): Effects of 6 week treatment with Microgynon and Nordiol on HDL level in female rats serum which were fed cholesterol rich diet. (No. = 6 rats in each group).

	HDL level (mg/dL)		
	Before treatment	**After treatment**	**P. Value**
Self control group	22.04±1.08	21.4±1.39	N.S
Microgynon group	22.04±1.08	14.73±0.79	P < 0.0 1
Nordiol Group	22.04±1.08	9.14±1.002	P < 0.01

Table (6): Effects of 6 week treatment with Microgynon and Nordiol on LDL and VLDL evels in female rats serum which were fed cholesterol rich diet. (No. = 6 rats in each group).

	LDL level (mg/dL)			**VLDL level (mg/dL)**		
	Before treatment	**After treatment**	**P. Value**	**Before treatment**	**After treatment**	**P. Value**
Self control group	21.66±3.56	22.24±1.87	N.S	10.09±0.37	10.94±1.01	N.S
Microgynon group	21.66±3.56	22.15±2.42	N.S	10.09±0.37	13.08±1.36	N.S
Nordiol Group	21.66±3.56	23.94±1.17	N.S	10.09±0.37	14.09±0.97	P < 0.05

The values expressed as mean ± SEM

Discussion: Effect on oxidative stress parameters:

Effect of Cholesterol Rich Diet: In this study cholesterol rich diet had no significant effect on GSH, MDA, levels; CAT and GST enzymes activities (P>0.05) in the serum of female rats and this is because hypertriglyceridemia does not cause oxidative stress(38) while hypercholesterolemia which is usually associated with oxidative stress(39) was not achieved in this study, hence there was no generation of reactive oxygen species or suppression of antioxidant system.

Effect of Microgynon Treatment: Serum GSH level decreased significantly (p<0.05) and this finding is consistent with that reported by Saroja *et al.*.(40) This may be due to low estrogen content in Microgynon (which has antioxidant effect). Serum MDA level did not significantly change (p>0.05) and this finding is in agreement with Kose *et al.,* who found that OCs with low dose LNG have no effect on lipid peroxidation.(41) Serum CAT activity did not significantly change (p>0.05), but Selda Kiranoglu *et al.,* found that OCs decrease CAT activity which may be related to the accumulation of H_2O_2(42), on the other hand Massafra C *et al.,* had found an increase in CAT activity during OCs treatment.(43) Serum GST activity did not significantly change (p>0.05) in Microgynon treatment, while Jendryczko A *et al.,* found that OCs decreased the activity of antioxidant enzymes.(44)

All these controversies may be due to differences in the used dose.

Effect of Nordiol Treatment: Serum GSH, MDA levels; CAT and GST activities did not significantly change (P>0.05), while Kose *et al.,* found that high dose of LNG in COCs causes lipid peroxidation and its effect on oxidative stress is not time but dose dependent.(41) This controversy may be due to the insufficiency of the dose used to induce such effect, and/or may be related to the high estrogen content in Nordiol that antagonizes the oxidative effect of LNG.

Effect on lipid profile: Effect of Cholesterol Rich Diet on Lipid Profile: A significant increase in serum TC level (P<0.05), TG, LDL and VLDL levels (P<0.01) in addition to significant decrease in serum HDL level (P<0.01) were found in rats fed a cholesterol rich diet as compared to the normal control. These results are consistent with that reported by Beynen A. C. *et al.,*(45) and Jale Balkan *et al.*(46) The further six weeks of cholesterol diet had no further significant effect on lipid profile.

Effect of Microgynon Treatment on Lipid Profile: Serum HDL level decreased significantly (P<0.01) while serum TC, TG, LDL and VLDL levels did not significantly change (P>0.05) in Microgynon treated female rats as compared with self control. These findings are in agreement with Kauppinen-Mäkelin *et al.*.(47) The molecular mechanism is that LNG stimulates hepatic lipoprotein lipase which was involved in the degradation of HDL, when a combination of EE_2 30 µg and LNG 150 µg is used. however, the effect of estrogen outweighs that of LNG on hepatic lipoprotein lipase resulting in a 30% increase. In spite of this, there was a significant reduction in the concentration of HDL.(47)

Effect of Nordiol Treatment on Lipid Profile: Nordiol treatment increased serum TG, VLDL levels significantly (P<0.05) and significantly decreased HDL level (P<0.01) while TC did not significantly decrease (P>0.05), these findings are in agreement with Godsland IF *et al.,*(48) and Burkman RT *et al.,*(49) the molecular mechanism for the significant decrease in HDL level is the same that for Microgynon treatment since both

contain the same ratio of EE_2: LNG which is 1:5.The significant increase in TG as suggested by La Rosa may depend on the estrogen component, which increases serum TG level because of increased VLDL production.(50) In this study serum, LDL level insignificantly changed (P>0.05). In contrast, Kose *et al.,* had found that serum LDL level increased by such treatment.(51)

This controversy may be due to the opposite effect of both estrogen and progesterone on LDL receptors stimulation, or the dose used may be insufficient to induce such effect.

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