



Alteration in Metabolic Cost of Blood Urea, Serum Albumin and Oxidative Stress Induced by Oral Contraceptive Pills (OCP) among Elite Females

**Aftab Ahmad Jan¹, Alamgir Khan^{2*}, Muhammad Zafar Iqbal Butt²,
Samiullah Khan³, Ejaz Asghar⁴, Muhammad Jamil⁵, Adnan Ahmad⁶
and Usman Muavia⁷**

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gomal University, Dera Ismail Khan, KPK, Pakistan.

²Department of Sports Sciences and Physical Education, University of the Punjab Lahore, Pakistan.

³Gomal Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, KPK, Pakistan.

⁴Department of Sports Sciences and Physical Education, ISRA University Islamabad Pakistan.

⁵Punjab High Police Lahore, Pakistan.

⁶Ideal High School, Kot Radha Kishan Distt Kasur, Pakistan.

⁷The city School, People Colony Branch Faisalabad, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. Authors AA, AK, MZA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript.

'Author SUK and 'Author MJ' managed the analyses of the study. Author AA, UM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: For controlling unwilling pregnancy, sustaining gorgeousness and good health, among the elite level female athletes the use of contraceptive pills is quite common.
Objective: Oxidative stress in female athletes is understudied. This research study was carried out in order to assess the alteration in metabolic rate of blood urea, bilirubin and oxidative stress induced by Levonorgestrel and Ethinylestradiol (oral contraceptive pills). The outcome of the study

will reveal the impact of oral contraceptive pills on the overall health of the females.

Methods: User and non-user of oral contraceptive pills were included as participants of the study. Sixty elite level female athletes using Levonorgestrel and Ethinylestradiol were recruited as experimental group and twenty four female (non-users) as a control group were taken as sample of the study. Blood sample (5ml) was taken from all the subjects. Blood urea and bilirubin metabolism were estimated through kidney functional test and for the measurement of oxidative stress FRAP assay was used. The data obtained through kidney functional tests and FRAP assay were processed through statistical package for social sciences (SPSS, Version 23.0)

Results: A significant difference was found between control and experimental subjects in FRAP values because ($t(82) = 3.236, P < 0.05$). The mean value of subject was 110.54 and control was 137.95 in FRAP the mean value of subject is less than the mean value of control ($509.3 < 700.7$). In case of bilirubin and serum albumin no difference was noted between control and experimental group as the value of significance is greater than 0.05.

Conclusion: Based on the analysis, the researcher concluded that oral contraceptive pills produced significant rise in oxidative stress, thus affecting the overall health of elite female athletes.

Keywords: Levonorgestrel; ethinylestradiol; contraceptive pills; oxidative stress, frap assay.

1. INTRODUCTION

Research evidence indicates that many of society's female practice oral contraceptive pills (OCP) for avoiding unwilling pregnancy and for maintenance of health. In addition, a long term health complication also associated with oral contraceptive pills. OCP are drugs or methods used to prevent unwilling pregnancy. It interrupts fertilization or implantation process [1,2]. Oral contraceptive pills contain two types of hormones i.e. estrogen and progesterone to avoid pregnancy by suppressing ovulation, thicken the cervical mucus and by blocking the sperm penetration [3,4]. Oral contraceptive pills containing estrogen and progesterone are similar to the hormone produced naturally by the female ovary. Use of contraceptive pills not only control the birth rate but is create many health problems for its users [5,6].

OCP cause oxidative stress and thus imbalance between reactive oxygen species and antioxidants accurse. Body is continuously exposed to various types of agents that results in the making of reactive species called as free radicals (ROS/RNS) which transfer of their free, unpaired electron causes the oxidation [7]. Effect of oxidative stress includes risk of deficiencies of carbohydrates, proteins, and lipids [8,9].

Natural antioxidant systems defuse free radicals formed due to biochemical reactions and protect the body from the poisonous effects of oxygen. Antioxidants are the substance that prevents the oxidation of organic molecules, synthesized in the body and taken with food [10,11]. The author further stated that maximum utilization or

consumption of oxygen can create free radicals in the body. This free radical action can be measured by electron spin resonance and by mitochondrial membrane damage, conjugated denies, short chain hydrocarbons and oxidized nucleosides.

During metabolic process, body naturally produced reactive oxygen species and antioxidants. Antioxidants control or minimize the reactive oxygen species. if this natural system is disturb due to any kind of internal or external factors than the body may lead to imbalance of reactive oxygen species and antioxidants and thus the body may lead to oxidative stress [12]. The author further stated that a number of cellular systems in the body cause the production of reactive oxygen species mainly in respiratory chain take place in the mitochondria. Normally we utilize 80 to 90 percent oxygen and out of this percentage some oxygen become changed to reactive oxygen species and in this way mostly the production of reactive oxygen take place through mitochondrial respiratory reactions [13].

Liver composed of a group of enzymes called cytochrome p 450 is present which is responsible for many functions in the body by removal and detoxification of compounds Present in our environment as well as ingested. Cytochrome p450 enzymes use molecular oxygen during the metabolizing of the compounds. Metabolism of these compounds by enzymes cytochrome p 450 generates reactive oxygen species [14].

Xanthine oxidase is oxidative enzymes present in cells of the body. This enzymes helps in the removal of hydrogen from xanthine by attributing this hydrogen to NAD and form NADH. During

unfavorable condition such as disturbed blood flow to a tissue xanthine oxidase, become changed to reactive oxygen species producing enzyme. In this way due to creation of reactive oxygen species, oxidative stress occurs [15,16,14].

Amino acid is the basic of protein and protein is the basic unit of cell, which perform a variety of functions in the body. More than 20 amino acid helps in the synthesis of protein. Due to different location and sequence each protein perform different function, these amino acids are sensitive to reactive oxygen species produces in the body in different situation and cause the inactivation of amino acids due to which protein become damaged and cannot perform it functioning normally [17].

Lipid, perform a variety of functions such as present in the cell membrane as well as in the nucleus and mitochondria [18,19]. Lipids are made up of un-saturated fatty acid due to peroxidation by reactive oxygen species cause the damage of lipids by attacking the double body of unsaturated fatty acids. In female using contraceptives there was significant increase in lipid peroxides where as such situation was not observed in women not using oral contraceptives [14,16,20].

OCP use might result in slightly inferior exercise performance on average when compared to naturally menstruating women. Oral contraceptive pills (OCPs) are double agents, which down regulate endogenous concentrations of oestradiol and progesterone whilst simultaneously providing daily supplementation of exogenous oestrogen and progestin during the OCP-taking days. This altered hormonal milieu differs significantly from that of eumenorrhic women and might impact exercise performance, due to changes in ovarian hormone-mediated physiological processes [21].

To explore the effects of OCPs on exercise performance in women and to provide evidence-based performance recommendations to users, there is an utmost need to perform research study to investigate the effects of OCP on oxidative stress and serum urea and serum albumin.

2. MATERIALS AND METHODS

2.1 Research Design

Because the research study was related with effects of OCP on blood urea, serum albumin

and oxidative stress among the female players, thus "experimental post-test only design" for representing the study in a reasonable and logical manner was employed.

2.2 Participants of the Study

Sixty elite level female athletes using Levonorgestrel and Ethinylestradiol (OCP) as experimental group (EXG) and twenty four female as a control group (CG) were taken as sample of the study. Subjects were included in the study through following criteria: a) subjects using contraceptive pills at least from last one year, b) subjects having no chronic health complication, c) subjects who voluntarily participate in the study and d) subjects not using any other kind of medication.

2.3 Blood Sample Collection

Blood sample (5 ml) was collected from each subject and transferred into serum accumulation gel tubes. Each tube marked with a subject distinguishing proof code. All subjects must finished a poll and an assent shape w marked by her.

2.4 Blood Urea Determination

Blood urea was determined by Urea UV diagnoses kit as described by [22] Four μ l reagent1 (R1), 100 μ l reagent 2 (R2) and 10 μ l blood serum was mixed in a test tube. Urease enzyme hydrolyzes urea into ammonia and then ammonium ions were estimated at 340nm by using UV-Visible Spectrophotometer (Microlab 300 Japan).

2.5 Determination of Serum Albumin

Serum albumin was determined according to spectrophotometric method [23].

2.6 FRAP Assay

For measuring oxidative stress FRAP assay was adopted by the researcher. The concentration of ferric tripyridyltriazine (Fe-TPTZ) compound decrease and convert to the ferrous form at acidic pH. A blue colored compound is observed at 593 nm. Degree of absorbance has been observed as to be directly associated to reduction of iron. To prepare the working FRAP reagents, TPTZ (2,4,6-Tripyridyl-s-triazine) and acetate buffer was mixed in 40 mM HCL as well as 20 mM FeCl₃.6H₂O in the ratio of 10:1:1 to

give the working FRAP reagent. In 300 mL of distilled water, 1.5 g sodium acetate was dissolved and added 8 mL of glacial acetic acid in 500 mL volumetric flask and volume was then made up to the mark with distilled water. The pH of solution was adjusted and stored at 4 °C. Thirty one mg TPTZ was added to 10 mL of 40 mM HCL and dissolved at 50 °C. Then 3.2 mL of conc. HCL (11M) was diluted with distilled water to 1000 mL in 1000 mL volumetric flask and volume was made up to the mark with distilled water after shaking it well. It was stored at room temperature. Fifty four mg ferric chloride was added in 10 mL distilled water and dissolved well and volume was made up to mark in 1000 mL volumetric flask. All other chemicals used in the solution were also of analytical grades.

2.7 Statistical Analysis of Data

The data obtained through Kidney Functional Tests and FRAP assay were processed through

Statistical Package for Social Sciences (SPSS, Version 23) by using different statistical tools.

3. RESULTS

Description of data of both groups (CG and EXG) in term of oxidative stress induced by OCP has been presented in Table 1. Similarly the data are articulated as mean and standard deviation etc. There is significant difference between control and subjects in FRAP because ($t(82) = 3.236, P < 0.05$). The mean value of subject was 110.54 and control was 137.95 in FRAP the mean value of subject is less than the mean value of control ($509.3 < 700.7$) as shown in Fig. 1.

The mean value of blood urea of EXG is ± 29.3 and the mean value of blood urea CG is ± 23.5 . As the value of sig. is greater than 0.05, it mean there is no difference between EXG and CG regarding blood urea as shown in Fig.2.

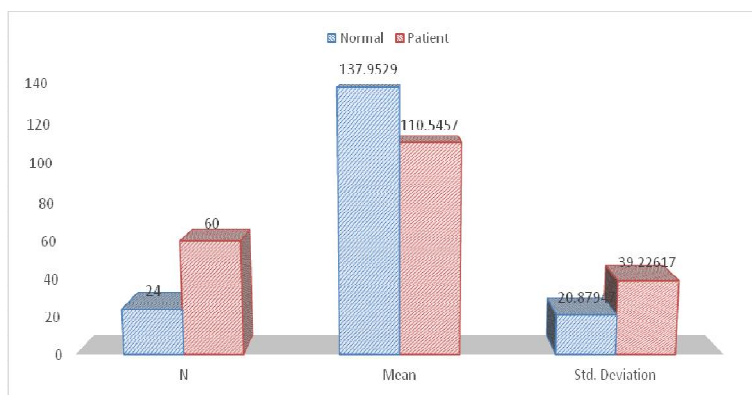


Fig. 1. Showing the comparison of oxidative stress induced by OCP in both Groups i.e. CG and EXG

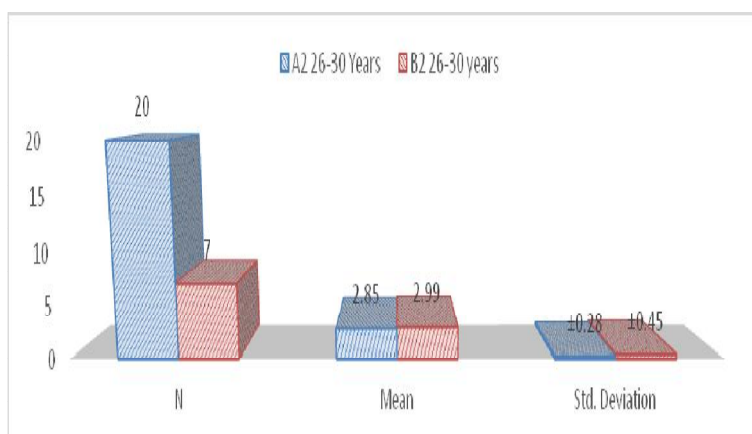


Fig. 2. showing the comparison of blood urea in both groups i.e. CG and EXG

Table1. Comparison of oxidative stress induced by OCP in CG and EXG groups of female athletes

Group statistics							
	EXG & CG	N	Mean	SD	Df.	T	Sig.
FRAP	CG	24	137.95	±.20.87	82	3.236	.002
	EXG	60	110.54	±.39.22			

Table 2. Comparison of blood urea of CG and EXG groups of female athletes

Group Statistics						
	Age based group	Number	M	S D	T	Significance level
Blood urea of both groups (EXG &CG)	A2 26-30 Years	20	29.3000	±8.48590	1.575	.128
	B2 26-30 years	7	23.5714	±7.59072		

Table 3. Comparison of serum albumin of CG and EXG groups of female athletes

Group statistics						
	Age wise differences	N	Mean	Std. deviation	T	Sig.
serum albumin mg/dl	A2 26-30 Years	20	2.8450	±.27999	-.975	.339
	B2 26-30 years	7	2.9857	±.44881		

The mean value of serum albumin in EXG is ±2.84 and the mean value of serum albumin in CG is ±2.98. As the value of sig. is greater than 0.05, it mean there is no difference between EXG and CG regarding serum albumin as shown in Fig. 3.

4. DISCUSSION

At present, the scientific interest in the effects of sport on oxidative stress levels is increasing both for health and performance implications. Several authors emphasize that additional research will be required in this field because discrepancies exist among studies especially regarding antioxidant strategies. Antioxidant and pro-oxidant pathophysiological pathways of physical activity need to be elucidated, apparently, beneficial effects on oxidative stress and health are obtained when regular moderate training is practiced. On the contrary, acute exercise seems to increase oxidative stress, but intriguingly the oxidative stimulus is necessary to up-regulate endogenous antioxidant defenses. Gender-specific studies are warranted to take into account sex differences in factors potentially modulating oxidative stress [24].

The study reveals that there is significant difference in both CG and EXG in FRAP value

because (t (82) = 3.236, P <0.05). The mean value of EXG was 110.54 and CG was 137.95 in FRAP, the mean value of EXG is less than the mean value of CG (509.3 <700.7). Such emerging concept is supported by [5] which indicate that oral contraceptive pills might cause oxidative stress. The author further stated that oral contraceptive pills not only cause oxidative stress but it can cause the failure of whole body functions. The findings of the present study indicate that female using oral contraceptives have elevated level lipid peroxides where as such situation was not observed in women not using oral contraceptives. Findings of the study conducted by [25] indicated that imbalance occurs in reactive oxygen species (ROS) and antioxidants due to use of oral contraceptive pills. It means that this findings also inline of the present study's findings. Elevated oxidative stress levels (≥310 FORT units) were found in 92.9 % of OC users and in 23.5 % of non-OC users (crude OR = 42, 95 % CI 12–149, p <0.001; adjusted OR = 60, 95 % CI 11–322, p <0.001). Continuous values of hydroperoxides were twofold higher in OC users versus non-OC users (median 484 versus 270 FORT units, p <0.001) and were inversely related to FORD units in OC users (p = 0.01) [24].

The study found that there is no difference in both CG and EXG regarding serum albumin.

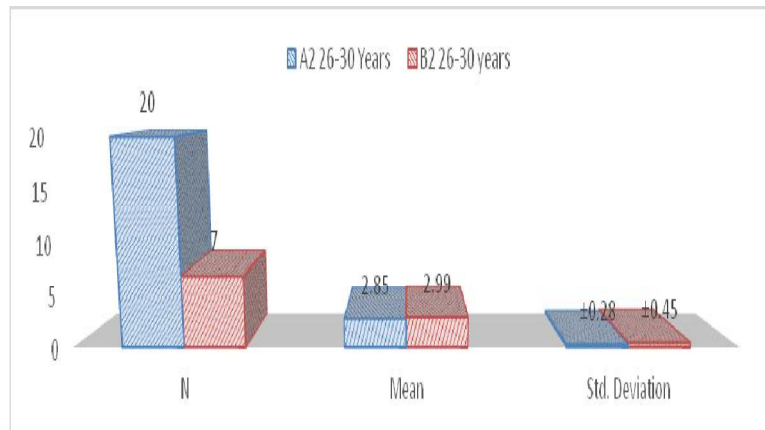


Fig. 3. Showing the comparison of serum albumin in both CG and EXG

Similarly no difference was found in EXG and CG in term of blood urea. The mean value of blood urea of EXG is ± 29.3 and the mean value of blood urea of CG is ± 23.5 . As the value of sig. is greater than 0.05, it mean there is no difference in both groups CG and EXG regarding blood urea. The mean value of serum albumin of EXG is ± 2.84 and the mean value of serum albumin of CG is ± 2.98 . As the value of sig. is greater than 0.05, so there is no difference between CG and EXP groups. Finding of the study conducted by [26] revealed that exercise positively influence the liver enzymes while OCP negatively influence the functional capacity of liver. Contraceptive pills influence the liver enzymes. Metabolic processes controlled by liver enzymes were found disturbed because of OCP [27].

Results showed significant increase ($p < 0.05$) in plasma creatinine, urea and K^+ but a decreases in plasma Na^+ and Cl^- in the tests compared to the control of female rabbits. Considering the observed changes in the parameters herein studied, COCPs usage is not without impact on kidney function and may cause homeostasis dysfunction and hence the need for further studies [28].

The female athlete triad (the triad) is an interrelationship of ... High-impact physical activity increases bone density in women, blood count, electrolytes, urea nitrogen, creatinine, glucose, calcium. The oral contraceptive pill (OCP) contains estrogen and progesterin and has few side effects [29].

5. CONCLUSION

On the basis of findings, the researcher concluded that OCP caused oxidative stress.

The oxidative stress may cause different kind of changes in hormonal system of the body as well. Additional research is desirable to extend our results, to clarify the biochemical pathways leading to increased hydro-peroxides (mainly lipid peroxides) and reduced antioxidant defense, and to elucidate the potential effects on athletic performance.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

All protocols of the study were approved by the ethical and research board of Gomal University, Dera Ismail Khan KPK, Pakistan. The written informed consent was taken from all the subjects before including in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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