



Ultra-structural Study by Transmission Electron Microscopy: Effect of Omega-3 on Ovary Cell Organelles after Experimental Induced Poly Cystic Ovary Syndrome

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Abstract

Objectives: Polycystic ovary syndrome (PCOS) is the most frequent cause of female infertility, affecting about 4% to 8% of women in the age of procreation. For evaluation of the protective effects of omega-3 polyunsaturated fatty acid on ovarian structure in experimental PCO induced by estradiol-valerate, this research was done.

Materials and Methods: Wistar female rats (n=40) were allocated into four groups, one control (n=10) and three test groups (n=30), that one group received omega-3 (60 mg/rat/orally/daily), second and third groups were induced PCO by single injection of estradiol-valerate (16mg/ kg/ i.m), third group also received omega-3 (240 mg/kg) for 60 consequence days. Animals were kept in standard conditions. On day 60, the ovarian tissue of Rats in whole groups were removed and prepared for pathological analysis.

Results: vacuolated area and rough endoplasmic reticulum expanded, de-granulated, disorganized were seen in PCO groups; however, these side effects decreased in the groups that received omega-3 significantly (p<0.05) in comparison to experiment groups and ovarian weights in PCO experimental decreased significantly (p<0.05).

Conclusion: Results revealed that administration of omega-3 could significantly treat PCO. This suggested that polyunsaturated fatty acid could diminish negative side effects of PCO on ovary tissue.

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Introduction:

Poly Cystic ovary syndrome (PCOS) is a common endocrine disorder in women during reproductive age (1). The prevalence of PCOS is estimated about 4% to 8% among reproductive-age women. PCOS manifestations include metabolic, reproductive, and psychological disorders. Thus, PCOS usually is along with many manifestations, including insulin resistance, hyperglycemia, hyperinsulinemia, type 2 diabetes mellitus, cardiovascular disease, hyperandrogenism, ovulatory dysfunction, infertility, increased anxiety, and depression. The phenotype varies depending on life stage of disease, genotype, ethnicity and environmental factors such as life style. The aetiology of PCOS is multifactorial probably. Diagnostic criteria rely on PCOS clinical and biochemical findings. Oxidative stress (OS) may play a role in the pathophysiology of PCOS (1,2,3) Normal hormonal levels in PCOS women converts to abnormal levels; the GnRH level changes indicate in most women with PCOS, LH levels are elevated and the LH/FSH ratio is high that can be considered as a diagnostic tool for this disorder (4). Evaluation of corticosteroid hormones demonstrates estrogen level increased in PCOs women while progesterone level decreased in these women (5).

Reactive oxygen species (ROS) are formed in the human body due to metabolism and cellular function; naturally, protective mechanism of the body against ROS is antioxidants. An antioxidant is a substance that can prevent or delay OS by scavenging biological important reactive oxygen species (O_2^- , H_2O_2 , OH, HOCl, ferryl, peroxy, and alkoxy) (6). Oxidative stress as a pathological state can be generated when there is an imbalance between pro-oxidant molecules (reactive oxygen and nitrogen species) and antioxidants defensive system. The role of OS in the pathogenesis of subfertility in both male and female has been shown by some studies; moreover, environmental pollutants cause OS, so the role of life style is prominent in generating oxidative stress. The impact of OS on oocytes and reproductive functions remains unclear.

OS may result in pathological diseases in female reproductive tract such as poly cystic ovary syndrome; hence, the antioxidants effect is very important to decrease infertility induced by oxidative stress (7). Omega-3, α -Linolenic acid is a poly unsaturated essential fatty acid obtained by nutrients (8). In the recent studies, the effects of n-3 fatty acids on the major risk factors of metabolic syndrome, especially adiposity, dyslipidemia, insulin resistance, diabetes, hypertension, oxidative stress, and inflammation have been assessed which show the beneficial effects of omega-3 on these parameters (9). The main purpose of this study is to assess the anti-oxidative and protective effects of poly unsaturated fatty acid (PUFA) on the ROS generated in PCOS induced rats.

Material & Methods:

Animals

Forty adult 8 week-old Wistar albino female rats weighing 250 ± 10 grams were obtained from Animal Facility of Pasture Institute of Iran. Rats were kept in temperature controlled rooms (25 C) with constant humidity (40-70%) and 12h/12h light/ dark cycle prior to use in experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care [NIH]. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz medical University. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed for each experimental animal. Thereafter, the rats were randomly selected and divided into control (n=10) and test groups (n=30), that subdivided into groups of 3, one group received omega-3 (240 mg/kg/orally/daily), second and third groups were induced PCO by single injection of estradiol-valerate (16mg/kg/i.m), third group of this research received omega-3 (240 mg/kg), for 60 days. Animals were kept in standard conditions. On day 60, the ovarian tissues of Rats in whole groups were removed for pathology analysis.

PCO induction

Thirty days before the experimental procedure, twenty rats were each given a single intra muscular (i.m.) injection of 4 mg EV (Riedeldehaen, Germany) solved in 0.2 ml oil in order to induce PCO (PCO group).

Surgical Procedure

On day 60, (at the end of the treatment period), the blood samples in control & experimental groups were immediately obtained.

Histopathology of Ovary

The ovarian tissues were fixed in 10% buffer formalin and embedded in paraffin wax. Five micron thick sections were obtained and prepared and then stained with masson's trichrome stain (T.C). The specimens were examined using an Olympus 3H light microscope.

Transmission electron microscopy

For transmission electron microscopy (TEM), the ovary samples were cut into pieces (2×2 mm) and fixed in 2.5% glutaraldehyde (PH=7.4) for 6-8 hr at 4°C. They were washed and post fixed in 2% OSO₄ for 1 hr, at 4°C. The tissue was dehydrated through ascending grades of ethanol and embedded in araldite CY212. Semi thin sections (1 μm) were cut and stained with toluidine blue. Ultra-thin sections (60-70 nm) were cut and stained with uranyl acetate and alkaline lead citrate.

Statistical analysis

Statistical analysis was done using the ANOVA test for comparison of data in the control group with the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). P-values less than 0.05 were considered significant and are written in the parentheses.

Results:

In the ovaries of the control group, thickness of zona pellucida and distribution of microvilli as well as the other organelles like mitochondria and rough endoplasmic reticulum were normal. Intercellular junction complex between corona radiata cells was intact. The nuclei of granulosa cells showed euchromatin appearance and prominent nucleolus. Normal mitochondria, rough endoplasmic reticulum and lipid droplet was obviously seen within the cytoplasm of granulosa cells. Following 60

days after inducing PCO by single injection of estradiol-valerate (16mg/ kg/ i.m), thickness of zona pellucida and distribution of microvilli was negatively affected and vacuolated area, rough endoplasmic reticulum was expanded, degranulated, disorganized, and even showed signs of rupture but intercellular junction complex was still observable. In PCO groups receiving omega-3, Nuclei of luteal cells were euchromatin with obvious nucleolus and still mitochondria were normal. Nuclear envelop dilation was the common finding of PCO damages which was seen after 60 days. Photomicrographs of ovary tissue in control group show normal structure of follicles, masson's trichrome staining, X160, ovary tissue in of ω-3 group show normal structure of follicle, masson's trichrome staining, X160. Other slide showed ovary tissue of PCO group cyst (arrow) and hyperemia (star), masson's trichrome staining, X160, figures:1,2,3.

Discussion:

Reactive oxygen species (ROS) and antioxidants are in balance in a healthy body. Reactive oxygen species is a phrase used to describe a variety of molecules and free radicals (chemical species with one unpaired electron) derived from molecular oxygen (10, 11). In the reproductive tissue, the elevated level of ROS generated by active metabolism and steroidogenesis may cause oocyte and DNA damage; besides, the ROS play a physiological role during ovulation that is similar to some responses to inflammation. ROS may play a role in the regulation of growth of ovarian mesenchyme in a pathological condition such as PCOs that excessive oxidative stress may contribute to ovarian mesenchyme hyperplasia. One of the protective mechanisms of the body against the ROS effect is antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) for eliminating the ROS. ROS can activate scavenging system and Redox system that could repair oxidized and damaged molecules by using NADPH as an original electron source (11, 12). The ROS cause lipid peroxidation, so it can damage DNA and/or change cell signalling and cellular function.

The oxidative stress (OS) leads to DNA damage of ovarian epithelium or cell apoptosis; However, oxidative status of the cell modulates follicular growth, corpus luteum formation, endometrial differentiation and embryonic growth. Oxidative stress may also be the cause of preeclampsia, abortion, endometriosis, PCOS, infertility, mole hidatyform, radical-induced birth defects; hence, evaluating protection against OS is very important in reproductive science.

Recent findings illustrated beneficial influence of oxygen radicals and ROS in many physiological states like development of germ cells in the uterine environment, oocyte maturation, ovulation, and corpus luteum function and regression. Oxidative stress (OS) may play a role in the pathophysiology of PCOS. OS may affect insulin resistance (IR) that is common in young non-obese PCOS women (11, 12, 13). ROS generation from mononuclear cells (MNCs) in response to hyperglycemia increased in PCOS independent of obesity. The resultant oxidative stress may contribute to a pro-inflammatory state that induces insulin resistance and hyperandrogenism in women with this disorder (14). Antioxidants as defensive mechanism of the body modulate the reactive oxygen species effect, and they also prevent oxidative stress. Enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT). Non-enzymatic antioxidants include ascorbic acid (Vitamin C), α -tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants.

There is a balance between both the activities and the intracellular levels of these antioxidants normally that is essential for the organisms health (15). In this study on rats we subdivided female rats to one control group and three experimental groups, first group just received omega-3, PCOS was induced by estradiol-valerate in second group (16 mg/ kg/ i.m) and third group was injected estradiol-valerate and omega-3; the experiment duration was 60 days. After 60 days pathological analysis

showed decreasing in thickness of zona pellucida and distribution of microvilli in the group injected estradiol valerate; in addition, in this group, expansion, degranulation and disorganization of vacuolated area and rough endoplasmic reticulum were obvious while in third group these findings decreased, mitochondria was normal and nuclei appearance of granulosa cells was euchromatin with prominent nucleolus.

This study could be used as an evidence for confirmation of recent studies about the omega-3 effect on ovary tissue (16). Superoxide dismutase (SOD) produced in cumulus oophorus cells is closely associated with oocyte maturation and also GPx and catalase are significant for reproduction health; moreover, GSH as a redox system exists in a large amount in the oocytes, so according to this study, it seems that omega-3 may modulate the ROS effect by affecting on these anti-oxidative systems but it is necessary more studies to confirm this idea (12,17,18). Superoxide (O_2^-), hydrogen peroxide (H_2O_2), and lipid peroxides are generated in luteal tissue during natural and prostaglandin-induced regression in the rat. The cellular origin of oxygen radicals in regressing corpora luteal is predominately from resident and infiltrated leukocytes, notably neutrophils. Despite physiological roles of oxidative stress, the higher production of these agents may lead to an increased risk of ovarian pathology and it would probably be exacerbated under conditions of reduced antioxidant status (19). Some studies indicated that oxygen radicals may function as intracellular regulators of steroidogenesis in the corpus luteum (20). Oxidative stress and depletion of the antioxidant glutathione (GSH) cause apoptosis in many systems. Previous works showed that antioxidants prevented apoptosis effectively (21). Apoptosis, a type of physiological or active cell death, has been implicated as a mechanism underlying regression of the corpus luteum (CL) in the rat, bovine, rabbit and ovine ovary. Reactive oxygen species play an important role in luteolysis in rodent ovaries (22). The decreased levels of Paraoxonase lactonase

(antioxidant enzyme), platelet-activating factor acetylhydrolase (antioxidant enzyme preventing LDL oxidation), but elevated asymmetric dimethylation (ADMA as an inhibitor of nitric oxid synthesis) might be a marker of development of atherosclerosis in the PCOS patients associated with insulin resistance (23). Paraoxonase 1 (PON1) as an enzymatic antioxidant prevents lipid peroxidation. According to some researches, there is a direct relation between increased malondialdehyde (MDA) and decreased antioxidant (PON1) activity and total antioxidant capacity (TAC). Since PON1 activity is lower in the PCOS women, dyslipidemia is common in these women; it may cause lower-density-lipoprotein (LDL) oxidation in arterial walls, so atherogenesis is also common in the PCOS women (24,25). Many studies about the effects of vitamin D and its derivatives on the hyperandrogenism, menstrual irregularity, infertility, insulin resistance (IR), and oxidative stress showed the therapeutic and protective effect of vitamin D3, 25 hydroxy vitamin D, and also calcium in the PCOS women (26-28).

The protective effect of vitamin E against oxidative stress has been shown in the improvement of cardiovascular diseases (29). α -Linolenic acid is an organic compound (carboxylic acid) found in many vegetable oils and fish oil; The other name of α -Linolenic acid is -cis-9,12,15-octadecatrienoic acid 18:3 (n-3). (three cis double bond) (30). Polyunsaturated fatty acids omega-3 (PUFA), particular eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), are bioactive lipids that positively impact on signalling pathways involved in the development of cardiovascular diseases, so cardioprotective action of PUFA omega-3 has been approved (31,32). Omega-3 may increase high density lipoprotein cholesterol (HDL), but it may decrease the triglyceride levels. In the women with PCOS, omega-3 can modify the adiponectin (a soluble matrix protein) level, insulin resistance, and lipid profiles (33,34,35).

Conclusion :

Supplementation with omega-3 fatty acids has beneficial effects on serum lipid

peroxidation and antioxidant enzymes in addition which may lead to decreased rate of occurrence of vascular complications in diabetes patients. The anti-inflammatory effect of n-3 fatty acids may also suppress the oxidative stress. Supplementation with antioxidants has been proposed to counteract oxidative stress and improve antioxidant status of the insulin resistance common in the PCOS women. In many studies, increased dietary PUFA intake was significantly associated with beneficial metabolic and endocrine effects in women with PCOS.

Conflicts of interest:

Authors declare that there is no any conflict of interest.

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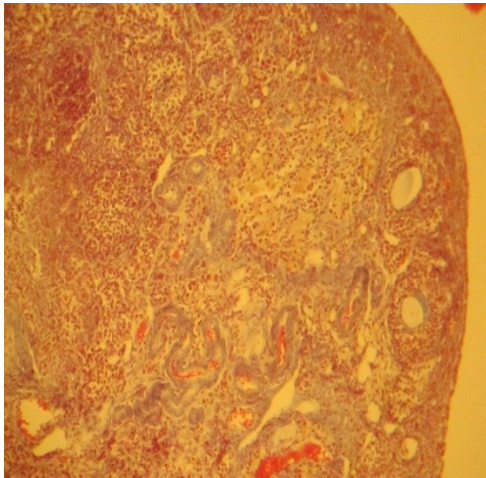


Figure 1. Photomicrograph of ovary tissue of control group shows normal structure of follicle, T.C staining, X160.

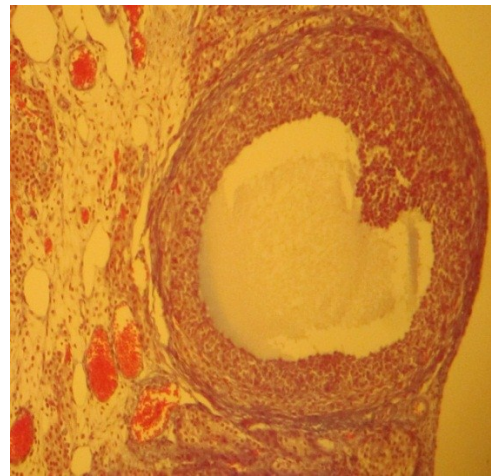


Figure 2 Photomicrograph of ovary tissue of ω -3group shows normal structure of follicle, T.C staining, X160.

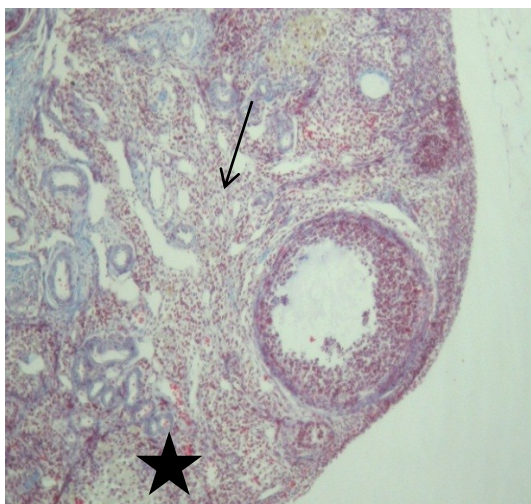


Figure 3. Photomicrograph of ovary tissue of PCO group shows cyst (arrow) and hyperemia (star), T.C staining, X160.

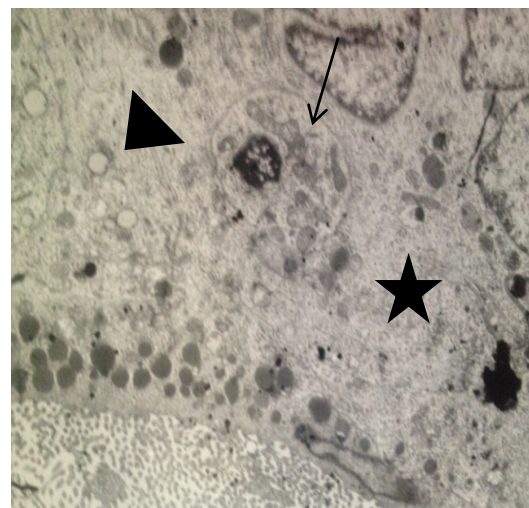


Figure 4. Electron micrograph of ovary tissue of PCO group shows cyst (triangle) and dilated mitochondria (arrow) and fibrosis .area (Star), X4000.

Table 1. Effect of Omega–3, fatty acids on ovarian tissue Fibrosis, cyst, mitochondrial dilatation and ovary weight of control in poly cystic ovarian (PCO) rats.

Groups	Control	Omega–3, fatty acids	PCO	PCO plus,1cc Omega–3, fatty acids /rat
ovary (gr)	1.09 ± 0.55	1.88 ±0.54	0.07 ±0.55*	0.09 ±0.55
Fibrosis (%)	0.00±0.55	0.00±0.01	3.11±0.55*	2.10 ±0.56*
Mitochondrial dilation (%)	0.00±0.05	0.01 ±0.10	6.70 ±0.55*	3.3 ±0.55*
Percent of cysts (%)	0.02±0.11	0.01 ±0.11	10.87 ±0.11*	5.01 ±0.11*

Data are presented as mean ± SE.

*Significant different at P< 0.05 level, (compared with the control group).

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