HISTOLOGICAL AND BIOCHEMICAL STUDY ON EFFECT OF THE ORAL CONTRACEPTIVE PILLS IN EXPERIMENTAL MALE MICE [+]

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Abstract :

The aim of this study is to find the effect of oral contraceptive pills(OCPs) on some biochemical parameters of the blood serum and histopathological changes in the testis and liver of the male mice.Eighteen adult male mice were divided randomly into edual three groups ; group I OCPs- untreated as control ,group II OCPs-treated with 0.00258g/kg daily for 15 days for biochemical study and group III OCPs- treated with 0.00258g/kg daily for 30 days for biochemical &histological study. The results showed that the oral contaceptive pills had caused significant increase ($P<0.05$) in GOT ,ALP enzymes and total cholesterol and significant decrease ($P<0.05$) in GPT enzyme , whereas total protein was not significanly changed. Histopathological changes were observed on testis as damage and destruction of seminal epithelial layer and redction of spermatogenesis, histolysis of interstitial tissue and damage in Leydig cells. Other histopathological changes were noticed in the liver including damage, hypertrophy in some cells and hyperplasia in different positions of other cells compared to control.

These results may suggest that OCPs had side effects on male fertility by damage of the spermatogenesis stages and Leydig cells which are responsible for producing testosterone hormone.

دراسة نسجية وكيموحيوية لدراسة تأثير حبوب مانع الحمل الفموي على ذكور الفئران المختبرية

وفيق حسن الاسماعيل

المستخلص:

تهدف هذه الدراسة معرفة التغيرات الكيموحيوية لتأثير حبوب مانع الحمل الفموي في ذكور الفئران البالغة من خلال معرفة تأثيرها على مستوى تراكيز بعض أنزيمات الكبد GOT و GPT وِALP ومستوى الكوليـــسترول الكلـــي Total Cholesterol والبروتين الكلي Total protein والتغيرات في التركيب النسيجي للخـــصية ومراحـــل تكـــوين النطف إضافة إلى تأثيرها على أكباد الحيوانات المجرعة بالعقار .

[+]**Received on 15/4/2012 , Accepted on 31/1/2013 .**

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أستخدم في هذه الدراسة 18 فارا ذكرا بالغا ، قسمت عشوائيا إلى ثلاث مجاميع متساوية ، المجموعة الأولى لم تعامل بالعقار واعدت كمجموعة سيطرة ، أما المجموعتين الثانية والثالثة عوملت بحبوب مانع للحمل وبجرعة 0.00258 غم/كغم لمدة 15 و 30 يوما على التوالي ، المجموعة الثانية لمعرفة التغيرات الكيموحيوية والمجموعة الثالثة لدراسة التغيرات النسيجية والكيموحيوية .

أوضحت نتائج الدراسة وجود ارتفاع معنوي ($p<0.05$) في قيم الأنزيمات GOT وALP ومستوى الكولستيرول وانخفاض معنوي ($P<0.05$) في قيم أنزيم GPT لمجموعة الحيوانات المجرعة بالعقار مقارنة مع حيوانات السيطرة . ولم يلاحظ أي فرق معنوي في تراكيز البروتين الكلي للحيوانات المعاملة مقارنة بحيوانات السيطرة .

أظهرت المقاطع النسيجية المأخوذة من خصى الحيوانات المعاملة بحبوب مانع الحمل الفموي وجود تحلل شامل لمعظم مراحل تكوين النطف وتفكك النسيج الخلوي للخصية وتحلل النسيج المحيط بالانبيب المنوي في مواقع مختلفة ، وانسلاخ وتلف خلايا لايدك Leydig Cells في مقاطع اخرى ، لوحظ في المقاطع النسيجية لأكباد الفئران المعاملة بالعقار، أن المعاملة أدت إلى تضخم في بعض الخلايا الكبدية وتحلل عظياتها ، وفي مقاطع أخرى شوهد فرط تنسج وتصبغ في بعض الخلايا الكبدية.

لذا فأن للعقار تأثيرا واضحا على الخصوبة من خلال تسببه في تلف مراحل تكوين النطف وتلف خلايا لايدك المسؤولة عن إنتاج هرمون التستوستيرون الذكري Testosterone.

Introduction:

The oral contraceptive pills (OCPs) are an increasing family planing method that has been used widely all over the world[1].

Combinations of hormonal preparation contain estrogen and progestin hormones that prevent pregnancy by inhibiting the pituitary gland and hypothalamus gland which inhibit the release of follicular stimulating hormone (FSH) and luteinizing hormone (LH).These hormones play key roles in the development of the egg and preparation of the lining of the uterus for implantation of the embryo .Progestin also makes the uterus mucinous that surrounds the egg make more difficult for sperm to penterate ,therefore fertilization does not take place [2]

Many studies indicated that the OCPs have some side effects such as weight gain,depression and low sexual desire[3].

Continuously using OCPs at a long time at middle- age leads to disturbance in cardiovascular system that increase the risk of thromobic events especially in the deep veins of the legs that caused deep venous thrombosis (DVT) .[4] .

Some studies domenstrated that the steroidal hormone contribute with the aggregation and distruction of blood platelets and the continuity of the young women at 20-24 year old for more than three years leading to low bone density .[5].

The relationship between breast cancer and short –term use of OCPs deserve further exploration especially the pills that have high dose of progesteron ,probabilty accurance of cervical and uterus cancer for women with OCP usage for more than two years .[6].

On other hand the uses of OCP lead to distrube liver function by increase the liver enzymes level especially Glutamate Oxaloacetate transaminase (GOT) ,Glutamate Pyravate Transaminase (GPT) and increase the level of cholesterol in blood ,whereas the Lovonogestral

compound elevate the level of LDL-cholestrol to 10% and triglyceride to 5% ,while the level of HDL-cholestral decreases among 5% .[7].

In addition women with a history of negative OCPs side effects had higher levels of eating disorder symptome which lead to increase the body mass from increasing protein synthesis ,reabsorpition of water and minerals because estrogen elevate the level of protein synthesis which effect on the growth of the body thickness .[8] .

Several studies have shown the increase of blood pressure in women whom uses OCPs more than 6ml/Hg result from effective of estrogen and the interferation between the OCPs and some drugs that used in chronic diseases such as analgesic ,antibiotics and heart diseases drugs [9].

Because of the distribution use of OCPs in some young male in viripotent stage in order to obtain clear white epidermic face , this study is suggested to know the side effects of OCPs on adult male fertility and the histolgical changes on testes , liver and liver enzymes .

Meterials and Methods:

1- The laboratory animals

A total of eighteen (18) adult male mice speases *Mus musculus* .L (each weighting approximately 25g) obtained from the Department of Biology , College of Scince ,Basra University .The animals were fed with standard diet and water, and were allowed to adapt to laboratory environment for two weeks. These animals were divided randomly into three groups , each group containing 6 mice ; group I was OCPs-untreated as control (which recived distilled water orally) ,group II was OCPs- treated with 0.00258g/kg for 15 days and group III was OCPs-treated with 0.00258 g/kg of OCPs for 30 days .

2- The drug used

Microgynon ED oral contraceptive pills which contain Levonorgestrel Fe 0.15mg and Ethinylestradiol 0.03 mg from Bayer Schering Pharma AG Germany was used according to Fenichel *et al.*, method [10] to determine the therapeutic dose that refer to take 0.015 part from pill weight that contain 0.0037 mg Levonorgestrel and 0.00075 mg Ethinylestradiol ,therefore recived 0.00258g/kg ; they diluted with distled water ,then administreted to the experimental animals daily for 15 or 30 days .

3-Biochemical study

After 15 and 30 days , the experimental animals were scarified by anesthetizing them in suffocating champer using chloroform ,blood was collected from the heart for separating serum with centrifuge speed 2000 r/m for 15 minutes to determine the liver enzymes GOT,GPT ,ALP and total protein and cholesterol ,using ready kits manufacterd from BioMerieux ,France .

4-Histological study

Testes and livers were romoved and immediately fixed in 10% formalin ,then transferred into an automatic tissue processor where they went throgh a process of dehydration in an ascending grades of alcohol (ethanol) 70,80,95 % and absolute alcohol for 2 changes each . Tissues were than cleared in xylene and embeded in parafin wax . Serial sections of 5 micron thickness were prepared using rotary microtome .

Tissue sections were deparaffinised ,hydrated and stained using the routine haematoxylin and eosin (H&E) staining method[11] .The stained sections were examined under light microscope type Nikon (x400 & x1000) .

5- Statistical analysis

The values were represented depending upon the analysis of variance test (ANOVA) using minitab program and revised least significant difference (R.L.S.D) .[12].

Results :

1-Biochemical Components

Table **1** shows the biochemical parameters for the mice administreted with OCPs for 15 and 30 days , These was a significant increase (P<0.05) in GOT enzyme level in mice treated for 30 days compared with control animals . Significant increase (p<0.05) in ALP enzyme values in mice treated for 15 and 30 days as well as in the levels of cholesterol in mice administrated with OCPs for 30 days . Significant decrease (p<0.05) in GPT serum enzyme in mice treated with OCPs for 30 days compared with control groups .

No significant changes in total protein level in mice treated with OCPs compared with control group .

Table 1. The biochemical parameters in mice treated with OCPs.

Group	GOT U/ml Mean±SD	GPT U/ml Mean±SD	ALP kAu/dl Mean±SD	Cholesterol mg/100ml Mean±SD	Total protein g /dl Mean±SD
Control GI N=6	2.301 ±0.081	21.376 ±0.699	12.392 ±0.342	46.900 ±3.82	4.426 ±0.691
Treated for 15 days G II N=6	3.723 ±0.296 NS	20.317 ±0.661 NS	**16.718 ±0.899	47.800 ±3.68 NS	5.633 ±0.703 NS
Treated for 30 day G III N=6	** 4.210 ±0.160	***17.620 ±0.712	**18.103 ±0.621	** 51.100 ±2.480	5.964 ±0.404 NS
Mice normal range	2.25-3.6	20-30	11-15	45 -48	4 – 6

□Significant Increase(P<0.05)in(R.L.S.D.)Test.
□□Significant Decrease(p<0.05)in(R.L.S.D.)Test .
NS No Significant changes .

2- Histological study

Morphological apperance of seminiferous tubules in verious shapes and sizes with stratified epithelium and normal spermatogonia ,spermatocytes ,spermatids ,spermatozoa and stertoli cells

in a well defined shape are shown in (Fig.1)which represents testes histological section from normal untreated control group mice.

In OCps-treated groups of mice ,histological section of testes reveal abnormal spermatogensis stages ,sloughing of the germinal epithelium from the basment membrane and damage of the soft tissue that surround the tubule (Fig. 2).On other sections there was some degrees of destruction of the interstitial tissue and damage in the Leydig cells (Fig. 3).

Concerning the livers histological sections of the OCPs-treated mice had demonstrated hypertrophy on some hepatocytes ,dissociation of some cytoplasmic organelles ,disappearance of the internal stored materials.

Hyperplasia and hyperchromatosis on some hepatocytes when compared with the livers of the control animals was also recognized (Fig. 4 and 5).

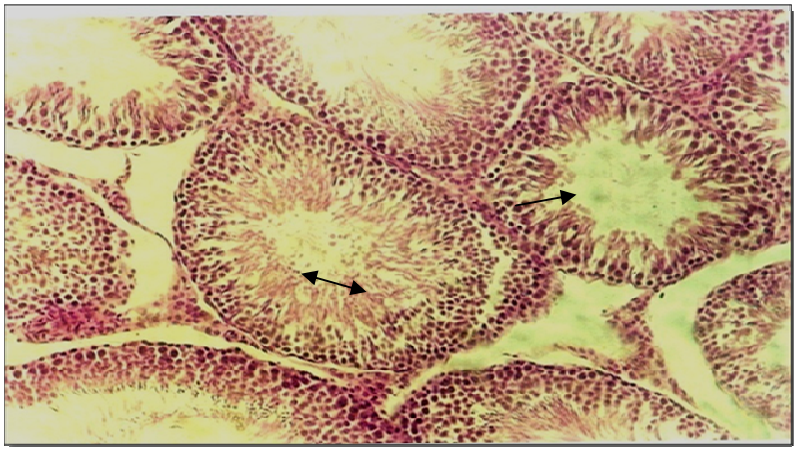

Fig.1 : Histological Section of OCPs-untreated control mice testis showing normal sperma togenesis stages(⟶) and normal spermatozoa (⟷). H&E.225x

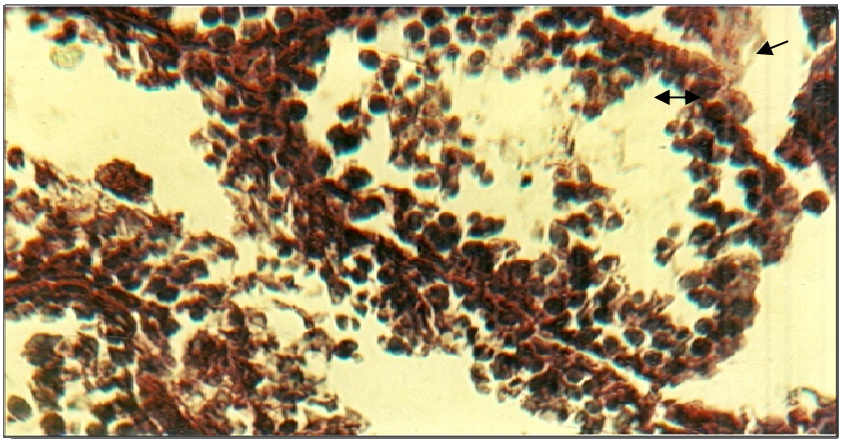

Fig.2 : Histological section of OCPs- treated mice testis showing complete histolytic of spermatogonia cells (⟷)and sloughing of germinal epithelial tissue (⟶ from basement membrane .H&E.750X.

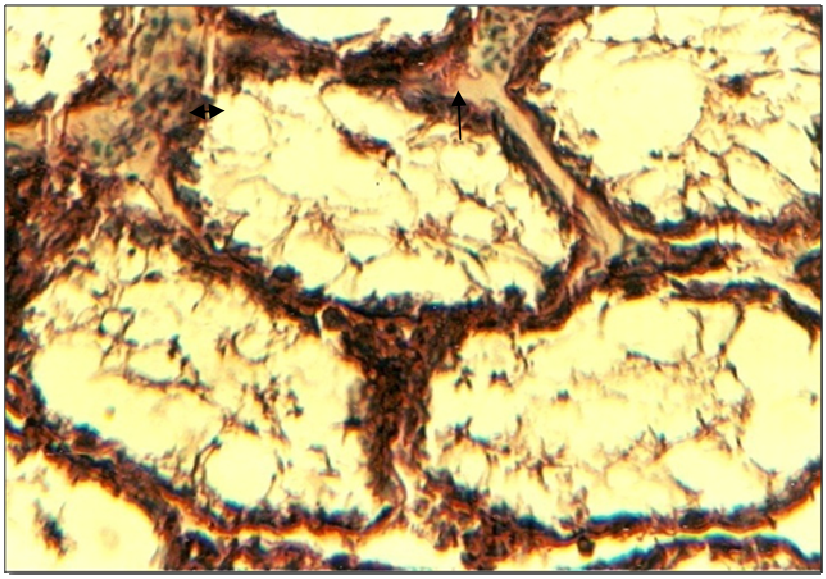

Fig. 3. Histological section of OCPs- treated mice testis showing complete histolytic spersp ermatogenesis stages(⟷) and damage of Leydig cells (⟶) ,H&E.750X

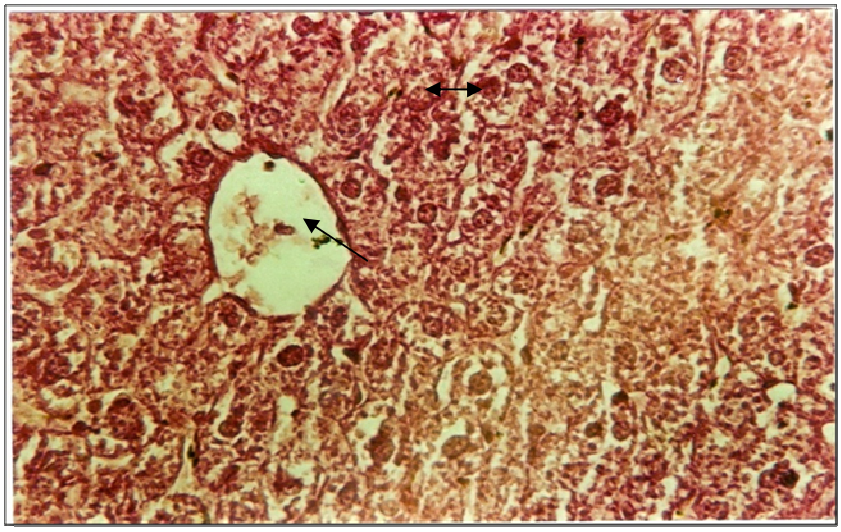

Fig. 4.Histological section on OCPs-untreated control mice liver showing central vein(⟷)and(⟶) hepatocytes.H&E.555X

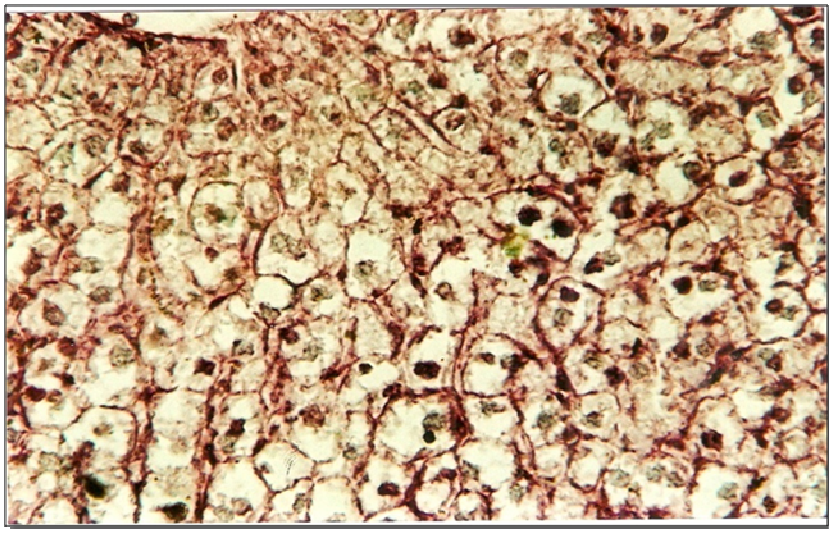

Fig.5. Histological section on OCPs-treated mice liver showing histolytic of hepatocytes cytoplasm (◄——►)and Hyperplasia (——►) H&E. 550X

Discussion :

In the present study the liver enzyme GOT expressed a significant increase level($p<0.05$),where as the liver enzyme GPT expressed a significant decreased level ($p<0.05$) in OCPs-treated mice compared to OCPs-untreated control mice .

These enzymatic changes may take place as a result of destruction of hepatocytes which are responsible for detoxication and their accompanying exodus the entrance enzymes to blood especially GOT enzyme which lead to their elevated levels in blood .These findings agreed with [13] which showed the significant increase in liver enzymes level after using OCPs ,while disagreed with[14] which refered that there is no significant increase in the levels of GOT,GPT enzymes in blood of animals treated with OCPs.

A significant increase ($p<0.05$)in the levels of serum ALP of OCPs-treated mice may reflect to damage hepatocytes, failure to release enzyme from bone ,intestine and liver because of infiltrative and granulomatous liver diseases [15].

Other observation was the significant increase ($p<0.05$) in cholestrol levels in animals blood treated with OCPs for 30 days compared with control group .These results agreed with [7 ,16] ,who clarified a significant incease in cholesterol levels in women's and animal's blood treated with OCPs for long time .

Total protein values of OCPS-treated mice had no significant diffrences compared with control. Recent observations also referred to that most progestins had no effect on protein metabolisum when given OCPs in order to prevent pregnancy[13].

Histological sections related to mice testes treated with OCPs for 30 days showed difference affections of drug ,which included destruction ,histolytic of spermatogonia cells and reduction in the number of spermatozoa in the central lumen of the seminiferous tubule ,which might be due to reduction of Leydig cells which are responsible for production of testosterone

hormone ; that influence from luteinizing hormone LH which are important in the formation of the sperms .Any destraction on it's function lead to disturbance in spermatogenesis processes [17]. These may be caused by decrease of cholesterol levels in the testis which lead to inhabition of endrogen scretions that has a role in sperm formation which depend upon the male accessary sex glands which are important on development of sperms [18].

These histopathological changes are inaccordance with the results of [19] where they suggest that the damage of the germinal epithelum in the testes leading to the spermatogenesis failure, and infertility could then appear .

Animal testis destruction after period of drug administration refer to hormonal drug effectivness because the male fertility controled by gonadotropins LH&FSH hormones and decrease level of these hormones which secreted from pituitary gland lead to functional failure in testes and formation of testosterone hormone which is an important hormone to spermatogenesis [20] .

Cell adhesion is important in the seminiferous epithelium .It is required for the nourishment of differentation of germ cells as they progress to the lumen ,loss of adhesion is another likely factor in cell damage and also lead to sloughing ,distorts histo-morphology and accelerates cell death [21].

The present study shows that OCPs treated to male mice for long period of time cause impaired spermatogenesis and histological damage to seminiferous epithelium . The study indicated that the histopathological changes on mice livers treated with OCPs for 30 days including damage and hypertrophy on some liver cells and hyperplasia in defferent positions of other cells compared with control group. The reason of cell damage may be caused by the effect of estrogen and progesterone on liver cells and hyperchromatosis cells result from necrosis and histolytic which lead to increase ability for more staining with starting of histolytic status [22]. Some types of OCPs verify on their affect on control at acne because they contain seroteron acetate which interfer with endrogen male sex activation and influence of lipid secretion in body that caused acne ,that limit from effectivness of male sex hormone by controlling on endrogen.

Conclusions:

The resuls shows a significant acute effect of OCPs on testicular structure .They are able to stop the spermatogenesis and necrotic tissue can be seen in testes .Other important finding that long period of administration of OCPs can damage spermatogenesis and can lead to fertility failure .The OCPs can also produce significant changes in the level of liver enzymes as well as some destructive changes in the hepatic tissue structure .

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