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The Histological And Genotoxic Effect Of Contraceptive Pills Administration On Oviduct In Female White Mice.

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ABSTRACT

The present work was designed to study the effect of contraceptive pills solution in white mice .The experimental animals were divided into four main groups (A,B,C, and D) and one control (E). Each group that composed of twenty animals .The experimental animals treated with the solution for one, two, three and four months with dosage (0.3ml) in concentration. The histological result of oviduct after 30 days showed decreased in ciliated cell. The secretory cells was flat and have a few secretory granules. The tissue result at 60 days showed increased in height of epithelia that lined of oviduct compared with control group. The epithelial layer of ampulla began lost the cilia. The histological result at 90 showed The low columnar epithelia turned to cuboidal in shape. Blood congestion seen in different part of oviduct section and a high vasculation seen along the epithelia layer. The result at 120 days showed the epithelia lost its cilia and the lumen of the secretory cells completely closed and full of secretions. The genotoxic level was increased after 30, 60, and 90 days (4, 6, 8, and 12% respectively).

Keywords: Oviduct; Cilia; Micronuclei; Contraceptive pills

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INTRODUCTION

The contraception means the ability to control fertility by reliable artificial methods has transformed both social and epidemiological aspects of human reproduction [1].The oral contraceptives (OC) are highly efficient and easily administered drugs; however, it must not be forgotten that they are composed of chemical substances which can be classified as potential carcinogens [2]. The Oral contraceptive pills is associated with the development of cervical cancer [3,4,5]. A systematic review of hormonal contraceptive use reported that the risk of in situ cervical cancer increase even for women with less than 5 years' hormonal contraceptive use, but the risk of invasive cervical cancer increase only after 5 years' use. The risk for both conditions declined with time since last use of hormonal contraceptives, and there was no elevated risk for invasive cervical cancer 10 years since the end of exposure [6].

Progesterone-only pills, commonly known as 'mini-pills' contain various types of progestogens and are used daily without a break. Most have a limited efficacy as contraceptive but were traditionally used as a contraceptive supplement to lactation. Prolonged exposure to progestagens induces endometrial atrophic changes. This an irregular and unpredictable bleeding pattern, often with reduced blood loss, duration, and menstrual frequency (occasionally amenorrhea) [7,8,9,10]. DNA is continually exposed to a variety of agents and processes (e.g., chemical carcinogens, ultraviolet and ionizing radiation, oxidative stress, and errors in DNA replication) that cause a range of DNA lesions (e.g., single-strand breaks, double-strand breaks, oxidative lesions, DNA adducts, and base mismatches, insertions and deletions). To preserve DNA integrity, cells have evolved mechanisms to detect DNA damage, signal its presence and mediate its repair [11,12,13].

MATERIALS AND METHODS

Animals group

The experimental animal divided into five main groups (A,B,C,D,E).The treated groups including (A,B,C,D).while the E group as control group. Each group composed of twenty laboratory animals. The treated groups divided according to the time of administration of the solution of contraceptive pills. Distributed of administration on 30, 60, 90, and 120 days. The constant concentration of oral contraceptive pills solution is (0.3 ml).

Group A .Treated with contraceptive pills solution for 30 days.
Group B. Treated with contraceptive pills solution for 60 days.
Group C. Treated with contraceptive pills solution for 90 days.
Group D. Treated with contraceptive pills solution for 120 days.

Oviduct histology preparation

Oviduct were removed and fixed in 10% formalin, tissues were then processed through different grades of thanol for dehydration. After that transferred into paraffin wax for 3 hours and placed into the molds prefilled with melted wax and cooled immediately at -60 °C to harden the wax. Tissues were then cut into 5 µm sections and mounted onto glass slide. The slides were then stained with hematoxylin and eosin (H&E).Visualized under a light microscope under magnification of (40x).(14) .

Micronucleus assay

After cleans the femur bone marrow lowered by using a sterile syringe to tubes contained 1ml of human blood serum then centrifuge by speed 1000 r.p.m to 5 minute. After that took a drop of sediment and do a smear on a clean slide .Left the slide overnight then dyeing by leishman stain. Prepared slides were examined by using oil immersion and counting the number of cell which contain micronuclei from 1000 cell. (15).

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RESULT AND DISCUSSION

The histological results of oviduct.

Histological result of the oviduct of control group:

The histological result of oviduct appeared that the mucosa of the ampulla exhibits the most extensive mucosal folds. These folds from an irregular lumen in the uterine tube that produces deep channels between the folds. These folds become smaller as the uterine tube nears the uterus (figure 1).The mucosa of the uterine tube consist of ciliated simple columnar epithelium that covers underlying connective tissue .The epithelium thickness was (14.527 ± 0.303µm) The muscular is layer consist of two smooth muscle layers, the inner circular layer and outer longitudinal layer. The interstitial connective tissue was abundant between the muscle layers. The smooth muscle layers especially the outer layer was not distinct.

Mucosal folds of the uterine tube lined by epithelium consist of ciliated and nonciliated cells. The ciliated cells most founded in the infundibulum and ampulla of the uterine tube. Under the epithelium was seen a prominent basement membrane. The lamina propria composed of ,loose connective tissue with fine collagen and reticular fibers .The histological result showed The mucosa of the oviduct presents a chains of longitudinal folds called plicae. Throughout the tube, the plicae consist of a single layer of columnar epithelial cells resting on an incomplete basement membrane and a lamina propria of richly cellular connective tissue that contains a complex of reticular fibers and fibroblasts [16].

Progressively, decreased through the the ciliated cells were most numerous on the surface of the fimbria an ampulla, isthmus, and intramural portions. The mucous membrane rests directly on the muscle coat. The muscle coat consists of two layers of smooth muscle cells. The inner layer was circular or closely spiraled, the outer layer was longitudinally. The muscular was increased in thickness to the uterus due to the increased peritoneal depth of the inner layer. Externally, the oviduct was covered by a serosa that represents the covering of the organ [17].

Treated groups

At thirty days :

The current study showed the tissue section of oviduct after 30 days have normal hight of epithelia with (14.343 ± 0.469µm) (Table1). compared with control group but noted small proliferation of epithelial cell in some location belong the epithelial layer of the oviduct. The present result appeared prominent basement membrane between epithelial layer of the oviduct with the underlying connective tissue. The epithelial layer of the ampulla of uterine tube was low ciliated columnar epithelia. The tissue result showed prominent basement membrane between the epithelial layer and underline connective tissue of the ampulla of oviduct with prominent muscular layers. This may be because of increased the thickness of epithelial layer that lead to disappear the cilia. This result coincides with [18] who demonstrated The epithelium lining the oviduct is simple columnar and consists of columnar ciliated cells. The current result after 30 days of administration of contraceptive pills solution caused a distraction of ciliated cell and a secretory cells. Cytoplasmic fragments of secretory cells were seen within oviductal lumen. The lumen of the secretory units became narrow and somewhat filled with secretion (figure 2). The oviduct was lined with a simple columnar epithelium .The lamina propria composed of loose connective tissue. The fibroblast was seen in lamina propria .Tunica muscular is thin and consist mostly of an inner circular layer and a few outer longitudinal layer of smooth muscle. This result was agreement with [18] who reported that the lessening in ciliated cells in affected fallopian tubes**.**

At sixty days :

The present study of tissue section of the oviduct after 60 days of administration of contraceptive pills solution showed significant increased in hight of epithelia with (21.081 ±0.677 µm) (Table1). compared with control group.The epithelial layer of ampulla began lost the cilia. the lumen of the secertory unit showed the narrow lumen and some of this contain the secretion this result may be due to the congestion in the blood vesseles that supply in the wall of the oviduct and the large amount of secretion that accumulation in the

secretory unit of the oviduct wall which lead to increase the hight of the epithelial layer .This result similar to [19] who showed epithelial height of fallopian tubes was found extensively increased (figure 3).

The histological result of the oviduct after 60 days of administration of contraceptive pills solution for white mice lead to increase in height of epithelium which lead to change the shape of epithelial cell. The ciliated cell seen in fewer amount and the secretory unit was fewer and its lumen was narrower than the control group. The lamina propria composed of loose connective tissue with thin fibers and numerous fibroblasts . This result were agree with [18] who decided that the ciliated cells are reported to have fewer and shorter cilia (figure 3).

At ninety days:

The histological result of oviduct after 90 days of administration of contraceptive pills solution showed the apical elongated nucleus at this stage may be because of the mitotic activity of the cells that composed of the layer of the oviduct. The low columnar epithelia turned to cuboidal in shape due to increase the thickness of the epithelial layer .The mean thickness of epithelia was (20.291 ±0.362 µm),(Table1).The current result showed proliferation in epithelial cells in some part of epithelial layer of oviduct. The current result showed after 90 days of administration of contraceptive pills solution for the white female mice which caused to blood congestion seen in different part of oviduct section and a high vasculation seen along the epithelia cell. The inflammatory cells also present. disappear cilia from epithelial layer and the secretory granules disappear and almost filled the lumen of the secretory units. This may be because of increased the thickness of epithelial layer that lead to disappear the cilia. This result agreement to the finding of [18] who showed the cilia decreased in affected tube (figure 4) (figure 5).

At one hundred twenty days :

The histological section of the oviduct after 120 days of administration of contraceptive pills solution showed significant increase in thickness of epithelia with (25.256 ± 0.403 µm) compared with control group (Table1). This may be because of hormonal effect on the astros cycle that lead to increase the amount of fluid in the lumen of secretory unit in oviduct wall which lead to increase the hight of epithelial layer. This result disagree with [20] who said the thickness of epithelia layer decreased. The current study after 120 day showed the epithelia lost its cilia and the lumen of the secretory unit completely closed and full of secretions. this may be because of increased the layer of epithelial cells that lead to disappear the cilia. This result was agree with [20] who said the cilia decreased in affected tube The blood congestion and blood spots also seen in histological section .The component of the lamina propria become lose their normal shape and sizes. The inflammatory cell increased and sever vacuolation showed in the cytoplasm of epithelia cell (figure 6).

Cytotoxicity result

Data of micronucleated erythrocytes in bone marrow cells of mice presented in below chart. The results show that at 30 days after administration of 0.3 ml of female oral contraception The bone marrow was affected in four percent. the control group was without any micronucleus. Our present result showed a mean micronuclei index count after 60 days of administration of contraceptive pills was be six percent. The present study showed increased amounts of micronuclei in third groups of mice which administrated for 90 days of contraceptive pills and the percentage of micronuclei formation became eight percent. The current result showed significant increased of micronuclei count after 120 days of administration of contraceptive pills which the percentage of micronuclei reached to twelve percent. Micronuclei primarily result from acentric chromosome fragments or lagging whole chromosomes that are not included in the daughter nuclei produced by mitosis because they fail to correctly attach to the spindle during the segregation of chromosomes in anaphase [21]. These full chromosomes or chromatid fragments are eventually enclosed by nuclear membranes and form a small nucleus is referred to as a micronucleus. Micronuclei can also form from fragmented chromosomes when nucleoplasmic bridges (NPB) are formed ,stretched, and broken during telophase. Micronuclei formation may also result from chromosome malsegregation during anaphase [22]. Hypomethylation of cytosine in centromeric and pericentromeric areas and higher-order repeats of satellite DNA in centromeric DNA can result in such chromosomal loss events. It has been suggested that sex hormones may modulate the activity of cytochromes p450 which are involved in the metabolism of

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endogenous and exogenous mutagens [23]. Another hypothesis concerns the mutagenicity of the hormones themselves. This result agree with [24, 25, 26] who said the values of MN significantly increase with time.

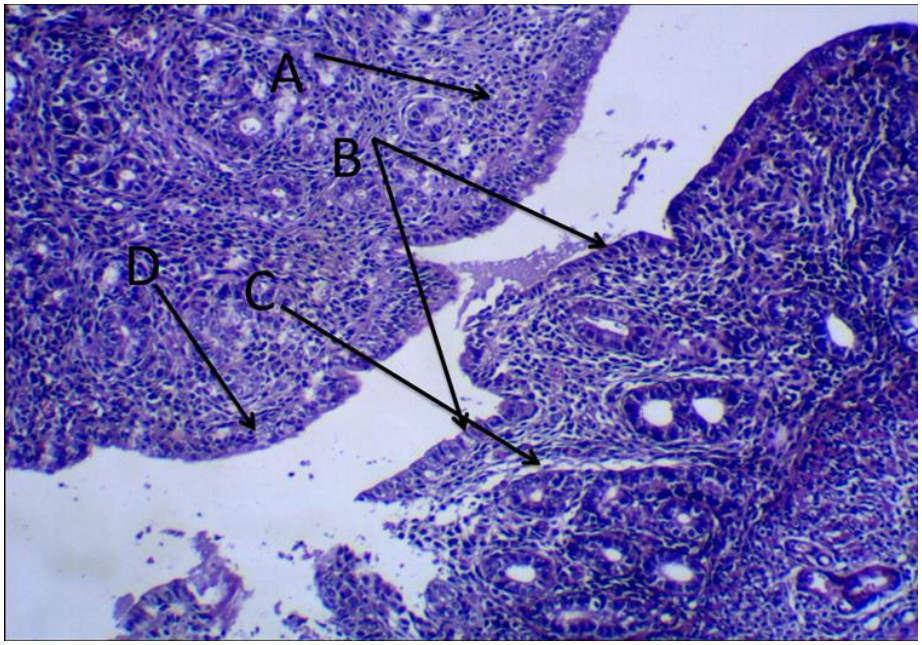

Figure 1. Transverse section through oviduct in control group which shows the A- Lamina propria, B- Mucosal fold,C- Lumen,D-Epithelium. H&E stain X100.

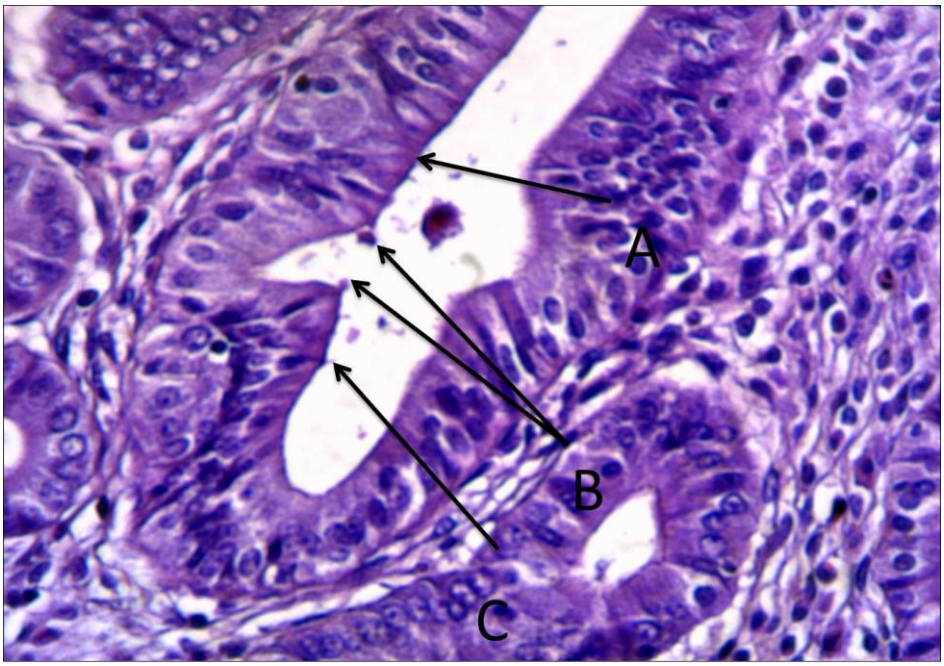

Figure 2. Transverse section through oviduct in treated group at 30 days of administration the oral contraceptive pills solution which shows the A- Low ciliated columnar epithelia , B- Secretory cell, C- Decrease ciliated cell. H&E stain X400.

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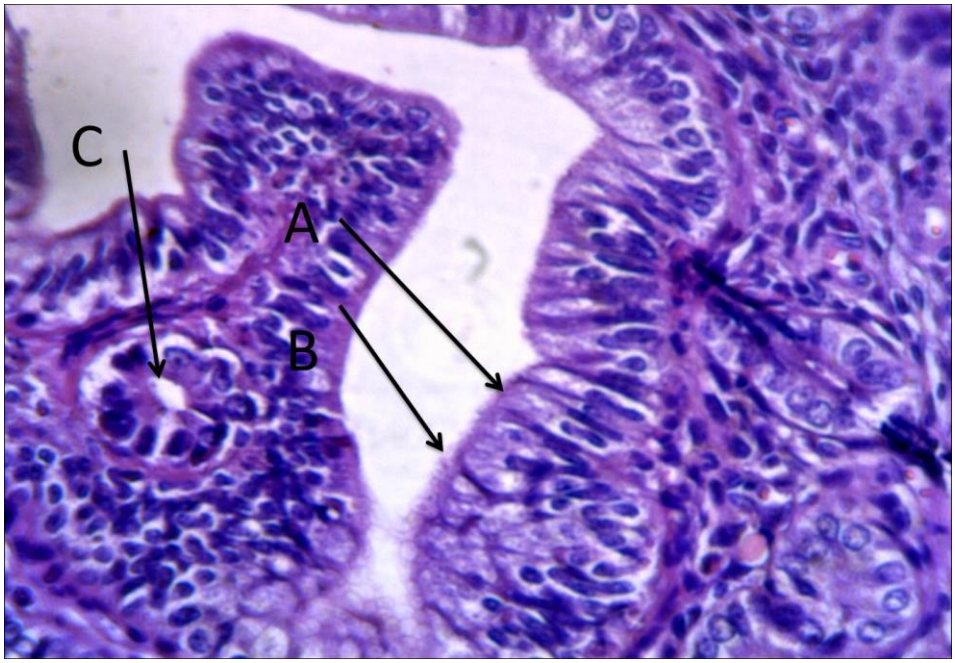

Figure 3. Transverse section through oviduct in treated group at 60 days of administration the oral contraceptive pills solution which shows the A- Increase hight of epithelium and became irregular, B- Decrease ciliated cell , C- Narrow lumen of secretory unit. H&E stain X400.

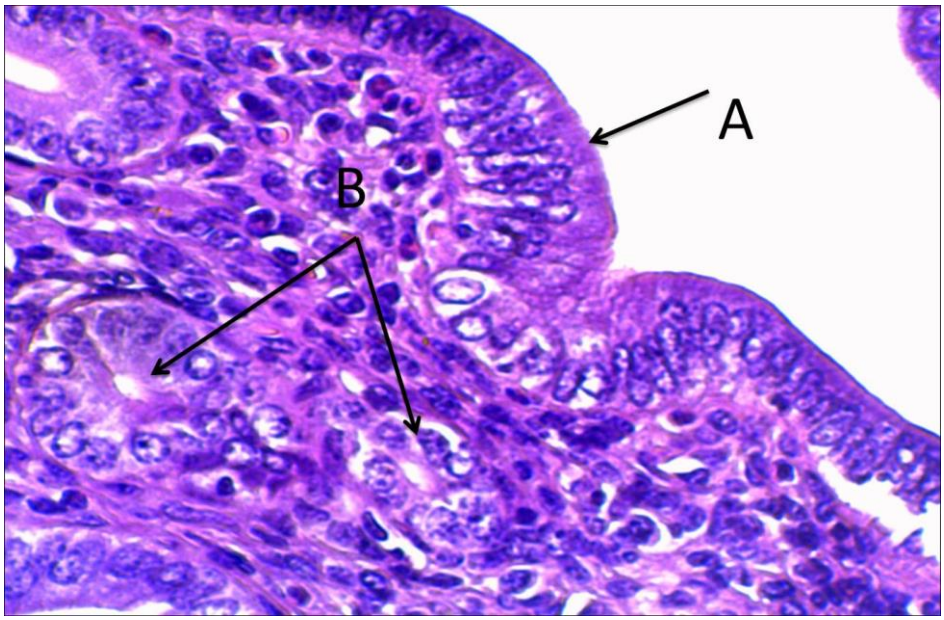

Figure 4. Transverse section through oviduct in treated group at 90 days of administration the oral contraceptive pills solution which shows the A- Disappear cilia from epithelial layer, B- Lumen filled with secretion. H&E stain X400.

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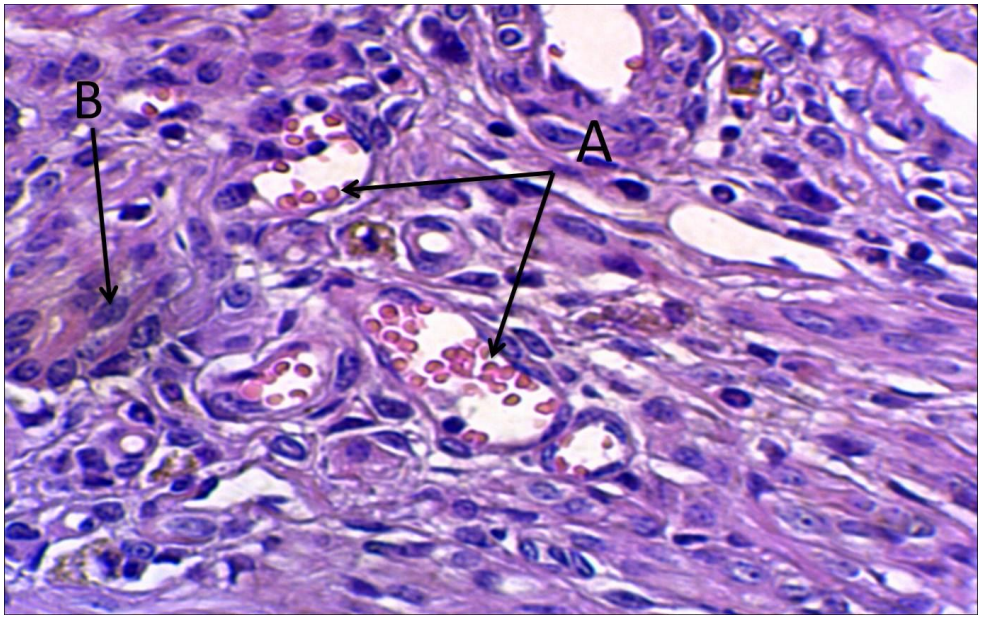

Figure 5. Transverse section through oviduct in treated group at 90 days of administration the oral contraceptive pills solution which shows the A- Blood congestion , B- Inflammatory cell . H&E stain X400.

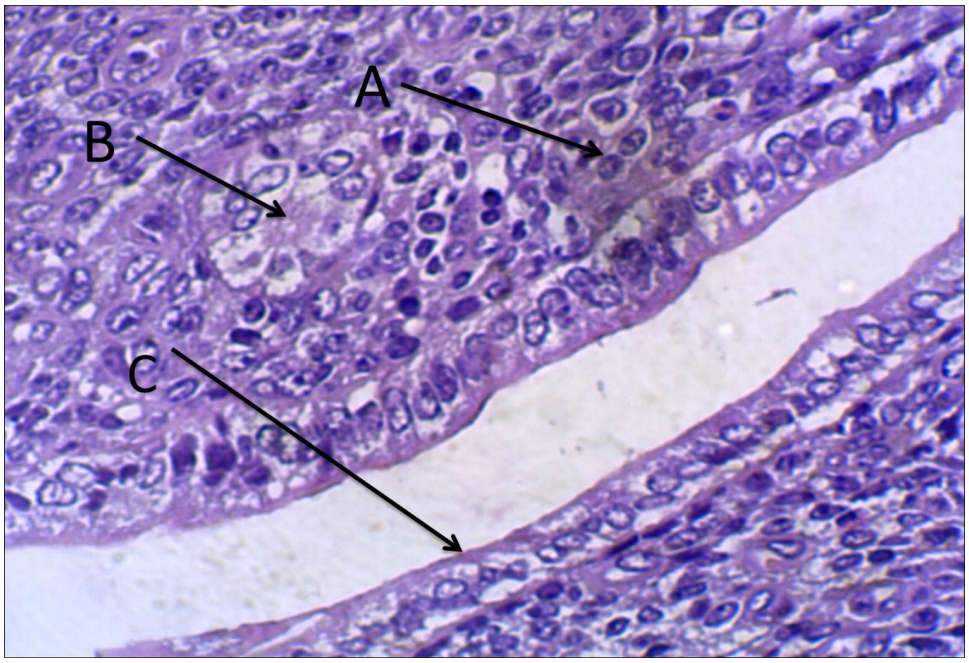

Figure 6. Transverse section through oviduct in treated group at 120 days of administration the oral contraceptive pills solution which shows the A- Inflammatory cell , B- Lumen of secretory unit closed, C- Cuboidal epithelia didn't have cilia. H&E stain X400.

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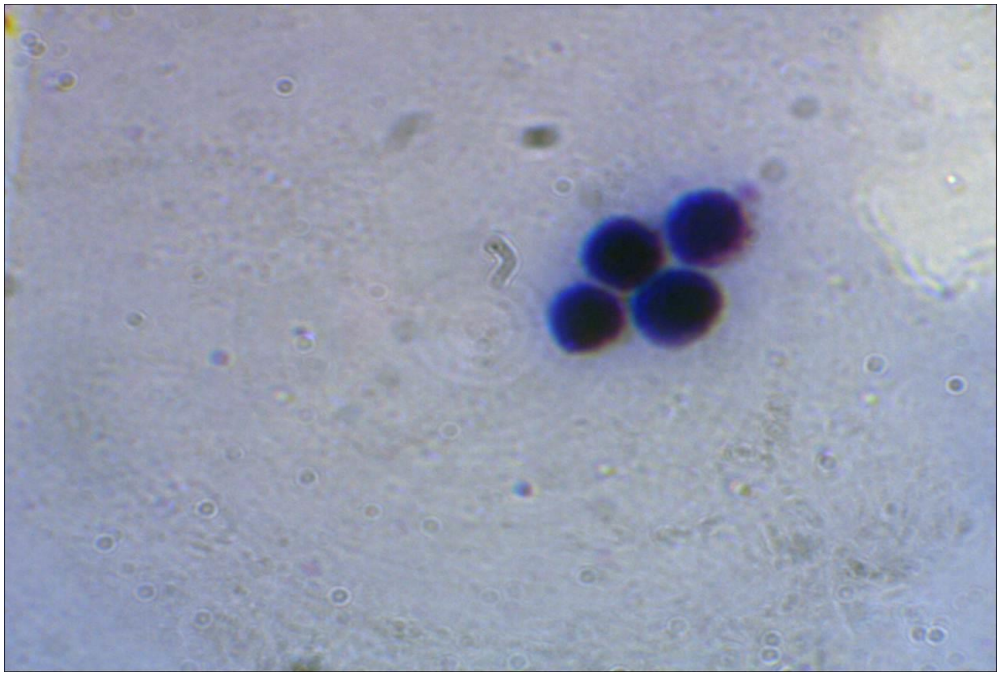

Figure 7. Control red blood cell without micronucleus. Stained by Leishman stain. 1000x

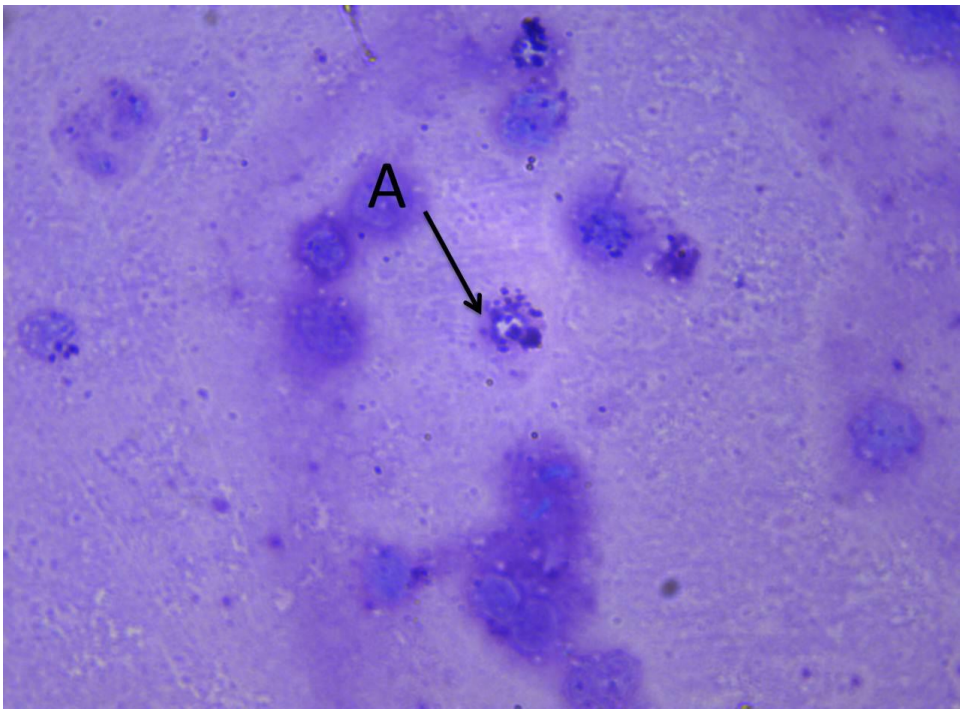

Figure 8. Red blood cell with micronucleus in treated group at 30 days of administration the oral 1000x. contraceptive pills solution which shows the A- micronucleus Stained by Leishman stain

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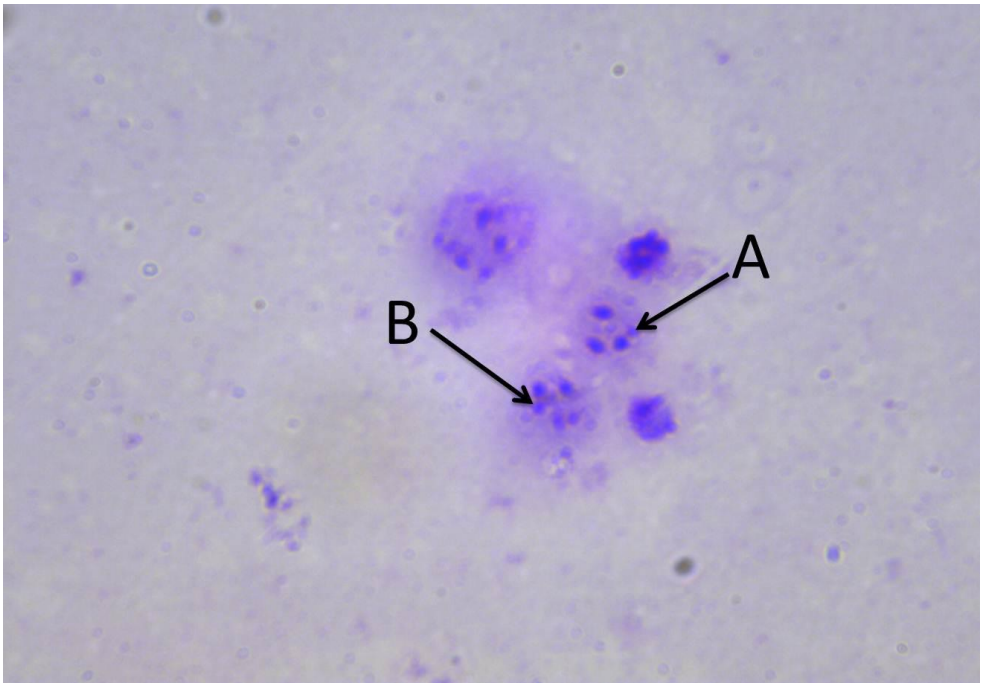

Figure 9. Red blood cell with micronucleus in treated group at 120 days of administration the oral 1000x. contraceptive pills solution which shows the A- micronucleus Stained by Leishman stain

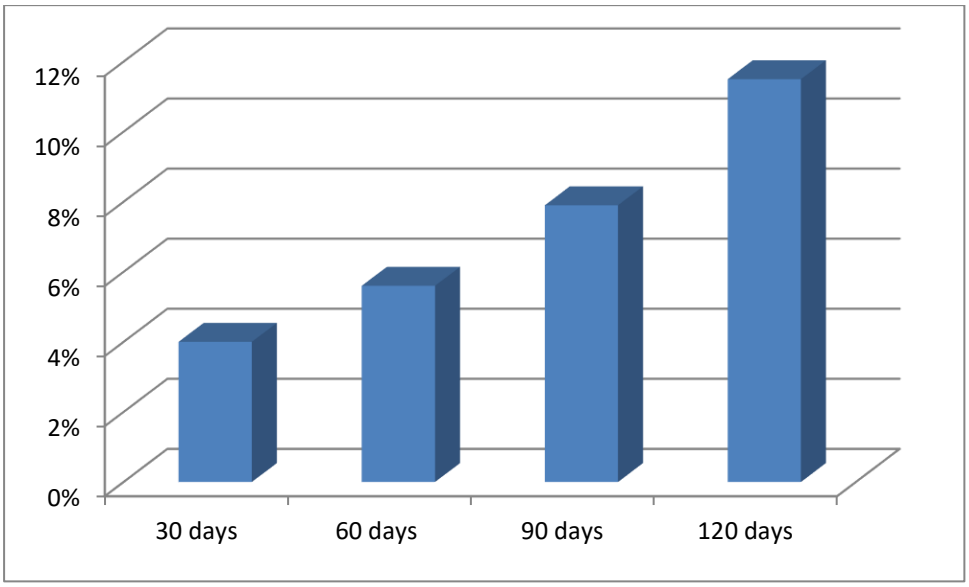

Figure 10. The percentage of micronucleus formation after oral administration of contraceptive pills solution on different days.

Table 1:Showed the epithelia thickness of oviduct.(µm)

	Epithelia Thickness of the oviduct
Control	14.527 ± 0.303 c
30 days	14.343 ± 0.496 c
60 days	21.081 ± 0.677 b
90 days	20.291 ± 0.362 b
120 days	25.256 ± 0.403 a

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CONCLUSION

It has been concluded that histopathologic examination of oviduct revealed that the prolonged used of oral contraceptive pills cause the epithelial layer of the ampulla lost their cilia this may be because of increased the thickness of epithelial layer of ampulla and caused the blood congestion and accumulation of inflammatory cells, the present study has revealed that there is a correlation of significant increased frequency of micronuclei formation and users of contraceptive pills .Amount of micronuclei increase with long term use of oral contraceptive pills. The micronuclei in bone marrow cells indicating strong cytogenic damage which may lead to cancerous proliferation.

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REFERENCES

[1] Ian M, Malcolm E, Contraception and sterilization. Essential of Obstetrics and Gynecology; 4th ed. 21 2004; pp:289.
[2] Bukvic N, Susca F, Bukvic D, Fanelli M, Guanti G, 17 α Ethinylestradioland Norgestrel in combination induce micronucleus increases and aneuploidy in human lymphocyte and fibroblast cultures. Teratog Carcinog Mutagen; 2000; (20): 147-159.
[3] Gao T, Wang J, Yang M, Li H, Transcriptome analysis reveals the effect of oral contraceptive use on cervical cancer. Mol Med Rep 2014; 10(4):1703–1708.
[4] La Vecchia C, Boccia S, Oral contraceptives, human papillomavirus and cervical cancer. Eur J Cancer Prev 2014; 23(2):110-112.
[5] Vessey M, Yeates D, Oral contraceptive use and cancer: final report fromthe Oxford-Family Planning Association contraceptive study. Contraception 2013; 88(6):678–683.
[6] Smith JS, Green J, Berrington de Gonzalez A, Appleby P, Peto J, Plummer M, Franceschi S, Beral V, Cervical cancer and use of hormonal contraceptives: a systematic review. Lancet 2003; (361):1159–1167.
[7] Saha PK, Rakshit BM, Jana N, Dutta S, Roy SB, Sengupta G, Management of abnormaluterine bleeding in women with mechanical heart valve prosthesis and anticoagulant therapy. J Indian Med Assoc 2011; (109): 908–911.
[8] Culwell KR, Curtis KM, Use of contraceptive methods by women with current venous thrombosis on anticoagulant therapy: a systematic review. Contraception 2009; (80): 337-345.
[9] Kadir RA, Chi C, Levonorgestrel intrauterine system: bleeding disorders and anticoagulant therapy. Contraception 2007; (75): 123-129.
[10] Pisoni CN, Cuadrado MJ, Khamashta MA, Hunt BJ, Treatment of menorrhagia associatedwith oral anticoagulation: efficacy and safety of the levonorgestrel releasing intrauterine device (Mirena coil). Lupus 2006; (15): 877–880.
[11] Lord CJ, Ashworth A, The DNA damage response and cancer therapy. Nature 2012; (481): 287-289.
[12] Basu B, Yap TA, Molife LR, De Bono JS, Targeting the DNA damage response in oncology: past, present and future perspectives. Curr. Opin. Oncol 2012; (24): 316-324.
[13] Jackson SP, Bartek J, The DNA-damage response in human biology and disease. Nature 2009; (461):1071-1078.
[14] Edward,G, Staining animal tissue. Practical 1st eds , leandard hill(Books), 1962 LTD.London.U.K.
[15] 15.Schmid W, The micronucleus test. Mutat.Res1975; (31): 9-15.
[16] Abe H, Hoshi H, Regional and cyclic variations in the ultrastructural features of secretory cells in the oviductal epithelium of the Chinese Meishan pig. Reprod Dom Anim 2007; (42): 292-298.
[17] Abe H, Hoshi H, Morphometric and ultrastructural changes in ciliated cells of the oviductal epithelium in prolific Chinese Meishan and large white pigs during the oestrous cycle. Reprod Dom Anim 2008; (43): 66-73.
[18] Zhao W, Mingxing Q, Cheng Y, Jiangjing L, Guojuan Y, Jian Q, Levonorgestrel decrease cilia beat frequency of human fallopian tubes and rat oviduct without changing morphological structure. Clinical and experimental pharmacology and physiology 2015; (42):171-178.

[19] Hegazy R, Hegazy A, Dmpa-induced changes in Estrogen and Progesterone Receptors of Ampulla of Rat oviducts: An Immunohistochemical study. Universal journal of medical science 2015; 3(2): 33-40.
[20] Okoko I , Oschiozokhai Y, Cytoarchitectural variations in the ovary, oviduct, and uterus following Intra-Gastric Gavages of Arbus Precatorius Linn in Albino Rats. Int.J.Morphol 2011; 29(4):1408-1413.
[21] Fenech M, Kirsch-Volders M, Natarajan A, Surralles J, Crott J, Parry J, Norppa H, Eastmond D, Tucker P, Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 2011; (26) 1: 125–132.
[22] Thomas P, Umegaki K, Fenech M, Nucleoplasmic bridges are a sensitive measure of chromosome rearrangement in the cytokinesis-block micronucleus assay. Mutagenesis 2003; 18(2):187-94.
[23] Geisler T, Geisler E, Schaeffeler J, Dippon S, Winter V, Buse C, CYP2C19 and nongenetic factors predict poor responsiveness to clopidogrel loading dose after coronary stent implantation. Pharmacogenomics 2008; (9): 1251-1259.
[24] Fenech M, Optimisation of micronucleus assay for biological dosimetry in: B. L. Gledhill, F Mauro (Eds.), New Horizons in Biological Dosimetry. New York. Wiley-Liss 1991; pp:373-86.
[25] Grujicic D, Effect of gestagen on the frequency of micronuclei in human lymphocyte of peripheral blood in vivo. Master thesis. University of Belgrade; 1999.
[26] Müller WU, Streffer C, Micronucleus assays. In: Advances in Mutagenesis Research, (ed. Obe, G.) Berlin; Springer-Verlag: 1990. pp:1-134.