Original Article

Effect of Lead Acetate on the Ovarian Growth in the Pre-Pubertal and Pubertal Periods in the Offspring of Albino Rats and the Possible Protective Role of Nigella Sativa Oil

Salwa M. Ouies[2], Salah R. Ahmed[1] and Asmaa S. Bassit[2]

[1]Department of Gynecology and Obstetrics, [2]Department of Human anatomy and Embryology, Faculty of Medicine, Sohag University, Egypt

ABSTRACT

Background: There are different responses of lead exposure including reduced fertility, spontaneous abortions, low birth weight, impairment in folliculogenesis, and even damage to the ovaries are also reported. Several reports confirmed the usefulness of black seed oil because of having more than one hundred of components as vol¬atile oil, vitamins and trace elements. Recently, clinical and animal studies revealed that the black seed extract has many therapeutic effects.

Aim of Work: To study the development of the ovary of the offspring of female rats exposed to lead acetate during pregnancy and lactation and the possible protective role of nigella sativa oil.

Materials and Methods: Three groups were used; Group A: Includes 15 offspring of 20 control mothers, Group B: Includes 15 offspring of 20 mothers treated with orally lead acetate in a dose of 640mg/kg, Group c: includes 15 offspring of 20 mothers in addition to the same dose of lead acetate each rat was taken orally nigella sativa oil in a dose of 10mg/kg. Both treatments were given to mothers from gestational day 10 to post-natal day 21.After the last dose the abdomens were opened and the ovaries were removed and processed for light and transmission electron microscopic study.

Results: Prenatal lead exposure caused Changes in ovarian architecture and severe pathological changes in the ovarian follicles in the form of delayed development of primordial follicles, damage to the granulosa cells and degeneration to the oocytes which appeared shrunken with vacuolated cytoplasm and destructed zonapellucida. Administration of nigella sativa oil prevents the damaging effect of lead on the ovarian follicles, still some oocytes and follicles preserved its normal appearance.

Conclusion: Exposure of mothers to lead acetate products cause harmful effects on the ovaries of the offspring, Administration of nigella sativa oil has ameliorative effects on these damaging effects of lead acetate.

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Key Words: Development, lead acetate, nigella sativa, ovary.

Corresponding Author: Salwa M. Ouies, MD, Department of Human anatomy and Embryology, Faculty of Medicine, Sohag University, Egypt, **Tel.**: +20 1002073124, **E-mail:** salwaouies@yahoo.com

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INTRODUCTION

The ovarian follicle is the functional unit of the ovary. The ovarian follicles are found within the stroma of the ovarian cortex. Follicles go through stages of development each month, with the goal of their maturation to release the oocyte for the purpose of fertilization and reproduction[1].

Maturation of oocytes begins before birth, once primordial germ cells (PGCs) have arrived in the gonad of a genetic female, they differentiate into oogonia, and they are arranged in clusters surrounded by a layer of flat epithelial cells,the flat epithelial cells, known as follicular cells, originate from surface epithelium covering the ovary. The majority of oogoniacontinue to divide by mitosis and arrested in prophase of meiosis I and form primary oocytes[2].

At puberty, a pool of growing follicles is established and continuously maintained from the supply of primordial follicles. Each cycle, 15 to 20 follicles selected from this pool begin to mature, passing through these stages in rats: primordial follicle, primary follicle, secondary or preantral follicles, tertiary or antral follicles (beginning of antral space formation) and preovulatory (Graafian follicle)[3].

The postnatal day (PND) 22 through PND 42 (or 43) is the period of pubertal development in the rat. End points in the female pubertal assay allow detection of test chemicals alter pubertal development via changes in luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin or growth hormone (GH) secretion, or alter hypothalamic neurotransmitter function. One of the required end points for this assessment is microscopic examination of the ovary at PND 42 or 43[4].

As ovulation approaches, the follicle enlarges and protrudes from the surface of the ovary. The thinning and increased distensibility of the wall facilitates the rupture of the follicle, followed by the formation of the corpus luteum[5].

Lead has become a regulatory concern and subject of much interest among pharmacologist, environmental scientist and clinicians because of its widespread distribution in environment due to its continuous emission

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from industrial sources and its pharmacological behavior to remain bound to mammalian tissues for a long duration[6].

It is well known that lead can pass through the placenta from mother to fetus, accumulates in fetal tissues during gestation and can be obtained through the milk during lactation so it exert their most serious adverse effects during fetal development[7].

Lead-induced reduction in number of primordial follicle and increase in number of atretic follicles have been reported in ovaries of mice[8] while in uterus, it damages endometrium, myometrium and perimetrium, along with reduction in uterine gland and decrease in height of columnar cells in mice[9].

The black seed Nigella Sativa (NS) is one of the natural anti-oxidant and anti-inflammatory agents. It also has many therapeutic effects such as immunomodulative, antibacterial, hypotensive, hepatoprotective and gastroprotective effects[10].

The efficacy of the NS oil is mostly attributed to its quinone constituents in the NS fixed and essential oil, which is especially endowed with thymoquinone (TQ), a significant bioactive constituent making up 30–48% of the total compounds[11]. Other functional components of the NS oil include p-cymene, carvacrol, thymohydroquinone (THQ), dihydrothymoquinone (DHTQ), α-thujene, thymol, t-anethole, β-pinene, α-pinene, and γ-terpinene. Among these, TQ has received the most attention and is mostly attributed to the learning and memory enhancing effects of NS. It has been shown to ameliorate diabetes-induced cognitive decline by preventing oxidative stress[12].

Several reports confirmed the usefulness of black seed oil as it has been used as a nat¬ural remedy to promote female menstruation, laxative and gastro protective in traditional medicineas well as bronchodilator activity and estro¬genic activity[13].

AIM OF THE WORK

To study the effect of lead acetate on the postnatal development(prepubertal and pubertal) of the ovary and the possible protective role of nigella sativa oil on albino rats through histological and transmission electron microscopic study.

MATERIAL AND METHODS

Materials

Lead acetate was brought from faculty of science as powder, nigella sativa oil was brought from market in the form of ampoule contain 20 ml.

Animals

90 albino rats; 60 adult females and 30 adult males, weighing 200-250g, were utilized for mating. These rats were obtained from the animal house of Assiut faculty of medicine. They were housed in Animal Facility at Faculty of Medicine, Sohag University, Egypt. All rats were given access for rodent chow diet and water. The experiment was performed according to the "Guide for the Care and Use of Laboratory Animals" (Institutes of laboratory Animal Research)[14] and in accordance with the guidelines of the University Animal Ethics and approved by Research Ethics Committee considering care and use of laboratory animals at Sohag University.

Experimental design

After a 7-day acclimatization period, adult females were housed with adult males at ratio of 2:1 respectively in each cage. Then, vaginal smears were taken in next day to detect presence of sperms. Pregnancy was calculated from the 1st day of the positive vaginal smear. Pregnant female rats were equally divided into three groups as follow:

Group A (control group): contained 20 pregnant rats which did not received any treatment.

Group B: contained 20 pregnant rats which received lead acetate

Group C: contained 20 pregnant rats which were received lead acetate and nigella sativa

Drug, dosage and administration

For group B Lead acetate in the form of powder 10 gm of lead dissolve in 100 cm of saline and each rat was taken lead acetate in dose 640mg/kg administrated orally using rats stomach tube every day from gestational day 10 to post-natal day 21[8], and for group C in addition to the same dose of lead acetate each rat was taken nigella sativa oil 10mg/kg using rats stomach tube every day from gestational day 10 to post-natal day 21[15].

Subdivision of groups

At birth, each mother was housed with its pups in a large cage in a ventilated room at a constant temperature (25°c.) with a12:12 h light/ dark cycle. Each group contained first generation of female pups with their mothers. Then, the pups were divided into three subgroups (5 female pups for each) according to the postnatal day of scarification as follow: Subgroups AI, BI and CI: sacrificed after 7 days postnatally (1 week) (PD7). Subgroups AII, BII and CII: sacrificed after 21 days postnatally (3 weeks) (PD21) Subgroups AIII, BIII and CIII: sacrificed at puberty age (6 weeks).

Methods

The animals were sacrificed 24h after the last dose; the abdomens open longitudinally and ovaries removed, formalin fixed and processed for normal Histological techniques, cutted in sagittal sections of 5 µm in thickness and stained with Haematoxylin and Eosin (H. and E.) Stain then examined by an Olympus light microscope[16].

Small pieces were also cut, fixed in 2.5% glutaraldehyde and processed for transmission electron microscopic examination(for 3 and 6 weeks)[17], semithin sections were stained with toluidine blue, sections were then examined by

an Olympus light microscope to choose the selected areas. Ultrathin sections, 50–80 nm, were cut from selected areas and examined in the transmission electron microscope unit, Assuit University.

Morphometric Study and Statistical Analysis

Estimation of the number of the ovarian follicles (primary, secondary, tertiary) healthy and atretic at the age of 1,3,6 weaks were done.

All previous parameters were measured using an image analysis system (Digimizer; Version 3.7. 2005-2010 Med Calc Software).

Variables were represented by mean ± Sd (Mean ± standard deviation of mean). The SSPS program version 16 was used to analyze the differences among all groups in all the data parameters by one-way analysis of variance and a post-hoc test was used to find the statistical difference between the groups when ANOVA was statistically significant (*P value* ≤0.05)[18].

RESULTS

The treated pregnant rats showed no external signs of toxicity, no mortality cases were recorded, no abortions; all the treated baby rats were survived to the end of study.

A-Light microscopic examination

1-Ovarian sections of PD7 albino rats (subgroups AI, BI and CI)

In control albino rats (AI),the normal architecture of the ovary at this age appeared; the cortex contained primordial germ cells and some primordial follicles; medulla appeared crowded with primordial and primary follicles and few secondary follicles (Figure 1) primary follicles appeared formed of primary oocyte surrounded by a single layer of cuboidal follicular cells, Secondary follicles appeared formed of primary oocyte surrounded by more than one layer of granulosa cells, Zonapellucida was presented between the oocyte and the adjacent follicular cells, Theca cells can also be identified in between follicles in the form of oval elongated cells with central nucleus (Figure 2).

In group BI: The ovary appeared shrunken with irregular outline and irregular distributed and damaged ovarian follicles (Figure 3), Primordial follicles were reduced in size and lost their normal distribution. Primary follicles appeared with destructed oocyte. Secondary follicles showed, oocytes not in normal shape and reduced in size, with dissolution in the cytoplasm and rarely seen with nucleolus, zonapellucida surrounding the oocyte was damaged in most of the follicles, disorder arrangement of the granulosa cells surrounding and the theca cells appeared disturbed and separated from the granular cells (Figure 4).

In group CI Ovaries appeared normally with intact surface epithelium, it had number of primordial follicles presented near the cortex and primary follicles present in the medulla, (Figure 5), primordial follicles showed normal appearance and central nucleus. Primary follicles appeared normal but few follicles appeared with destructed oocyte, most of the secondary follicles showed normal oocytes but some appeared abnormal, theca cells showed normal appearance in-between the follicles (Figure 6).

2- Ovarian sections of PD 21 albino rats (subgroups AII, BII and CII)

At PD21 of control albino rats (AII); Follicles development was progressively established so that simultaneously small, medium and large follicles are very clearly seen and in proper architecture. Primary, secondary and tertiary follicles were found along with zonapellucida, oocytes showed proper nucleus with darkly stained nucleolus and multiple layers of granulosa cells were also visible. Graafian Follicle (Pre-ovulatory Follicle) was appeared (Figure 7). In semi thin sections oocyte appeared with preserved cytoplasmic material, eccentric nucleus with central nucleolus and surrounded by zonapellucida also granulosa cells appeared normal clear with prominent nucleus (Figure 8).

At BII subgroup: The ovary showed irregular surface epithelium with different types of ovarian follicles, most of them appeared with damaged oocyte; primary, secondary and tertiary follicles. Multiple atretic follicles appeared within the ovary (Figure 9).In semi thin sections the granulosa cells were irregularly distributed and darkly stained with an area of destructed cells, there was thinning in the zona pellucida surrounded abnormal oocyte and the theca cells were disorganized and separated from each other (Figure 10).

CII subgroup the ovaries had intact surface epithelium, numbers of primary and secondary follicles still had destructed nuclei, tertiary and graafianfollicles appeared normal with normal granulosa cells and oocytes (Figure 11). In semi thin sections secondary follicle appeared with normal oocyte which had well defined nucleus with nucleolus surrounded by well-developed zonapellucida with normally appeared granulosa cells and intact theca cells outside them (Figure 12).

3- Ovarian sections of pubertal albino rats (subgroups AIII, BIII and CIII)

The Ovariansections of the 6 weeks Control rats (AIII) showedthat the ovary became differentiated into peripheral cortical and central medullary zone, the follicles were mainly located into the cortical region. They included different types offollicles; multiple corpus lutei appeared at this stage. The medulla was consisted of connective tissue stroma containing many blood vessels. Atretic follicles were also present. (Figure 13). In semi thin sections the oocyte appeared normal with preserved cytoplasmic organelles surrounded by smooth regular zonapellucida, the surrounded granulosa cells appeared normal with observed nucleus forming corona radiatia around the oocyte (Figure 14) .

At BIII subgroup: The ovary had follicles that mainly located in the cortical region, most of them showed damage

granulosa cells and shrunken nuclei, multiple atretic follicles and areas of hemorrhage appeared in-between the follicles, the medulla consisted of connective tissue stroma containing many dilated blood vessels, (Figure 15) In semi thin sections the oocyte had faint nucleus and destructed cytoplasm surrounded with a thin zonapellucida,the granulosa cells surrounded it were shrunken with darkly stained nucleus and multiple empty spaces in-between (Figure 16).

At CIII subgroup: The ovaries appeared with different follicles mainly located into the cortical region; included Primary follicle, secondary follicle and tertiary follicle, some follicles appeared normal and othersappeared with shrunken oocyte, some atretic follicles were located within the cortex (Figure 17). In semi thin sectionsthe oocyte were near normal with normal surrounding zonapellucidaand surrounded by normal granulosa cells but areas of destruction were still present (Figure 18).

B- Electron microscope study

1-PD21 albino rats (subgroups AII, BII and CII)

Ultrastructural examination of 3 weeks control rats (AII subgroup) showed:granulosa cells containing large nuclei surrounded by regular nuclear membrane, cytoplasm contained mitochondria and rough endoplasmic reticulum (Figure 19), the zonapellucida surrounded oocyte was smooth and intact, the cytoplasm of oocyte showed preserved organelles mitochondria, free ribosomes and rough endoplasmic reticulum (Figure 20).

At BII subgroupthe granulosa cells appeared shrunken with destructed cytoplasm and loss of cytoplasmic organelles and darkly stained nucleus, also multiple vacuoles appeared in-between these cells (Figure 21). Thezonapellucida surrounded oocyte showed irregularity, the cytoplasm of the oocyte showed multiple vacuolesand scanty nuclear organelles (loss of ribosome and rough endoplasmic reticulum, mitochondria also appeared shrunken and dark (Figure 22).

At CIIsome granulosa cells appeared with normal nuclei and cytoplasm, others appeared with darkly stained shrunken nucleus and loss of cytoplasmic organelles with appearance of vacuoles in the cytoplasm (Figure 23). The zonapellucida surrounded the oocyte was smooth and intact and the cytoplasm of oocyte showed preserved organelles (Figure 24).

2- 6 weeks albino rats (subgroups AIII, BIII and CIII)

At subgroups AIII the granulosa cells of the control animals showed normal appearance with large nuclei limited by regular nuclear membrane, the cytoplasm showed multiple rounded mitochondria and intact ribosomes. (Figure 25), oocyte surrounded by intact and smoothzonapellucida,its cytoplasm contained rounded mitochondria, intact rough endoplasmic reticulum and free ribosomes (Figure 26).

At subgroups BIII granulosa cells showed different sizes and appeared with darkly stained and shrunken nuclei with loss of surrounded organelles, there were multiple vacuoles in-between the granulosa cells. (Figure 27), the oocyte appeared surrounded by thin and irregularzonapellucida, the cytoplasm showed scanty destructed organelles (some mitochondria, few rough endoplasmic reticula, and free ribosome) (Figure 28).

At subgroups CIIIsomegranulosa cells appeared with normal nucleus and cytoplasm, others had shrunken nucleus with loss of cytoplasmic organelles. Some appeared with normal nucleus and destructed cytoplasm (Figure 29), the zonapellucida surrounded the oocyte appeared normal, the cytoplasm of the oocyte appeared with preserved organelles (mitochondria,ribosomes and rough endoplasmic reticulum) (Figure 30).

Morphometric results

Number of the normal and atretic follicles

1-At the age of 1 week

- The mean number of normal follicle in group t1 was (6.1) which was very high significantly decreased ($P \leq 0.001$) compared to the control group (15.7) and in group t2 was (12.1) which was very high significantly decreased compared to control group ($P \leq 0.001$) (Table 1, Histogram 1).
- The mean number of atretic follicle in group t1 was (6.5) was very high significantly increased ($P \leq 0.001$) compared to the control group (1.7) and in group t2 was (3.9) which was very high significantly increased compared to control group ($P \leq 0.001$). (Table 1, Histogram 1).

2-At age of 3 weeks

- The mean number of normal follicles in group t1 was (17.7) which was very high significantly decreased (P= 0.000) compared to the control group (28.2) while group t2 was (22.2) which was highly significant decreased compared to control group ($P \leq 0.001$). (Table 1, Histogram 2).
- The mean number of atretic follicles in group t1 was (15.4) which was very high significantly increased (P=0.000) compared to the control group (3.7),in group t2 was (11.3) which was highly significantly increasedcompared to control group ($P \leq 0.001$) (Table 1, Histogram 2).

3-At age of 6 weeks

- •The mean number of normal follicles in group t1 was (7.9) which was very high significantly decreased (P= 0.000) compared to the control group (11.6)in group t2 was (10) which was significantly decreased ($P \leq 0.01$)compared to control group (Table 1, Histogram 3).

- The mean number of atretic follicles in group t1 was (8.1) which very high significantly increased ($P \leq 0.001$) compared to the control group(4.3) While in group t2 was (5.3) which was significantly increasedcompared to control group ($P \leq 0.01$) (Table 1, Histogram 3).

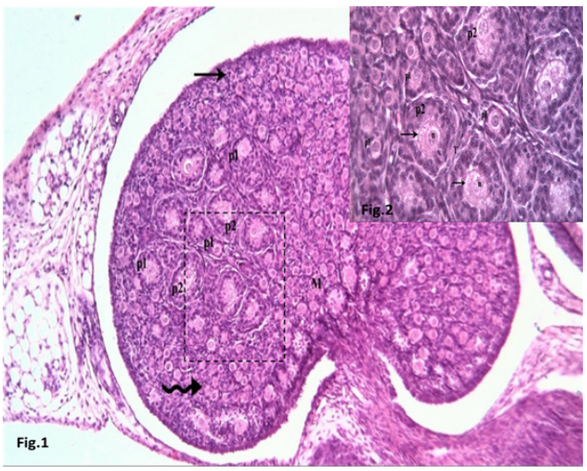


Fig. 1: photomicrograph section in the Ovary of control rats at PND 7showing normal structure of the surface epithelium with abundant primordial germ cellsclose to it (arrow) and the inner medulla (M) appears crowded with Primordial follicles (irregular arrow); Primary follicles (P1) and secondary follicle(p2) clearly appear in the center. H & E X100.

Fig. 2: Magnified part of the previous ovary showing; Primary follicles (P1)with a layer of cuboidal follicular cells and central nucleus, secondary follicle (p2)with multiple granulose cells and oocyte with eccentric nucleus (n) and layer of zonapellucida (arrow).Theca cells can also be identified (T) in between follicles . H & E X400.

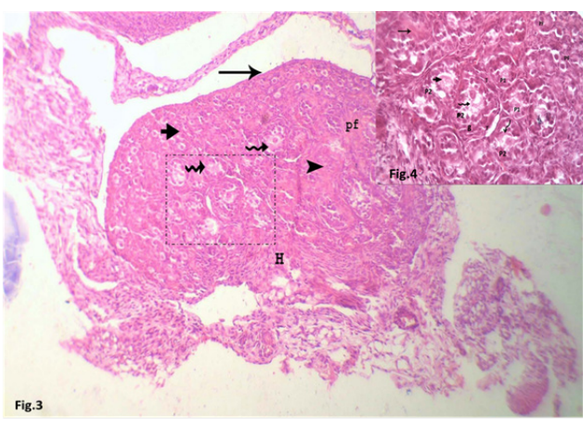


Fig. 3: photomicrograph section in the Ovary of lead treated rats at PND 7 showing irregular surface of the ovary with reduction in size (longarrow), persistence of multiple primordial germ cells (short arrow), Primordial follicles (Pf) are reduced in size and lost their normal distribution. Also, there is disarrangement of the primary follicle (irregular arrow),area of fibrosis appears (head arrow) close to the helium of ovary (H). H & E X100.

Fig. 4: Magnified part of the previous ovaryshowing;area of disarranged granulosa cells (long arrow),Primordial follicles (Pf) are reduced in size and lost their normal distribution,distorted primary follicle (P1) appearing with damaged oocyt,secondary follicle (p2) showingdamaged oocyt (short arrow), destructed zonapellucida (irregular arrow) and disarranged surrounding granulose cells ,theca cells (T) appear disturbed and separated from the granulose cells. H & E X400.

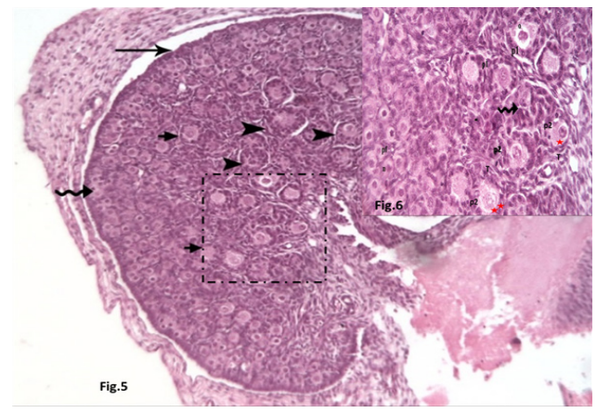


Fig. 5: photomicrograph section in the Ovary of lead andnigella sativa treated rats at PND 7showing normal appearance of the surface epithelium (long arrow). Primordial germ cells(irregular arrow) appear close to the surface, multiple primordial follicles (short arrows) and Primary follicles (head arrow) with normal appearance are visible at medulla. H & E X100.

Fig. 6: Magnified part of the previous ovary showing; the primordial follicles (pf) appear normal and central nucleus (n), most of the primary follicles(p1) has normal shape but some of these follicleshave shrunken oocyte(o),most of the secondary follicles(p2) showing normal oocytes(star) but some appear abnormal (double star), area of disorganized granulosa cell (irregular arrow) appears,theca cells (T) show normal appearance.H & E X400

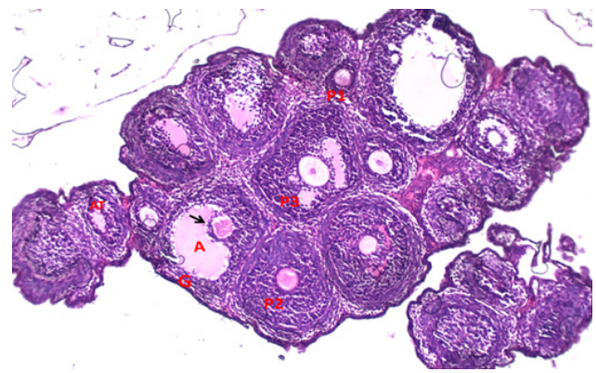


Fig. 7: photomicrograph section in the ovary of 3 weeks control rats showing, primary follicles(p1), secondary follicles (p2), tertiary follicles (p3), and Graafianfollicle(G) with large space or antrum(A),cummulusoophorus surrounding the oocyte (arrow)atretic follicles also appear (AT). H & E X100.

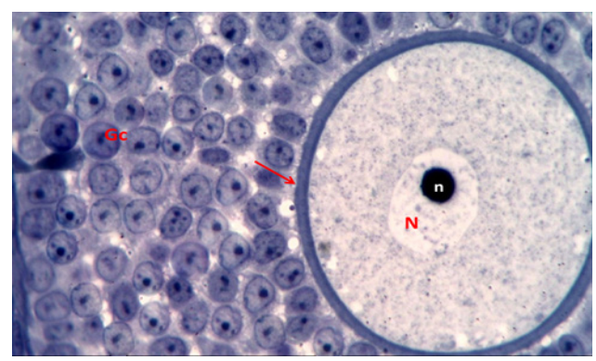


Fig. 8: A photomicrograph of semi-thin section in the ovary of 3 weeks of control rat showing part of an oocyte of secondary follicles appears with well- defined nucleus (N) and prominent nucleolus(n) surrounded with well-defined zonapellucida (arrow), the granulosa cells appear with normal shape (Gc).Toluidine blue; X1000.

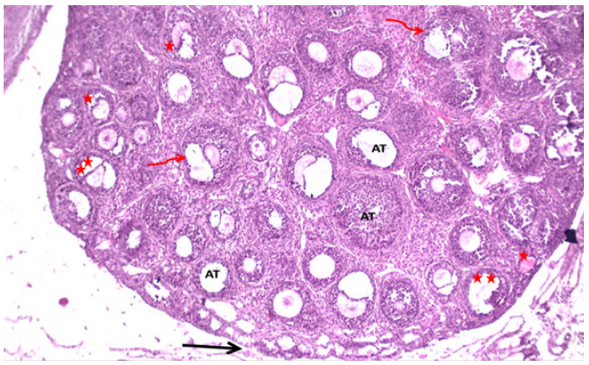


Fig. 9: photomicrograph section in the ovary of3 weeks lead treated animals showing detached and irregular abnormal surface epithelium (arrow) , primary follicles with damaged oocyte without nucleus in some areas (star), secondary follicle with destructed oocytes (2 stars),tertiary follicles with irregular granulosa cells and vacuolated oocyte and irregular zonapellucida(irregular arrow), multiple atretic follicles also appear (AT). H & E X100.

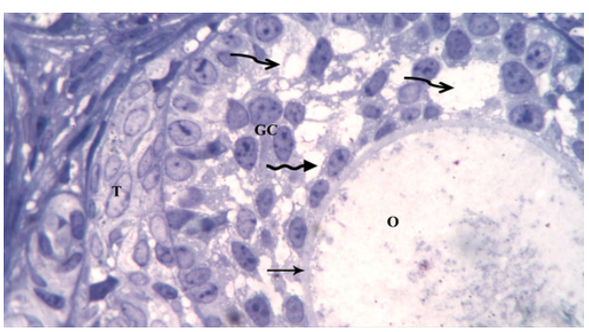


Fig. 10 : A photomicrograph of semi-thin section in the ovary of 3 weeks lead treated rat showing part of an oocyte(o) of secondary follicle with its surrounding thin zonapellucida (arrow), surrounded by irregular dark stained granulosa cells (GC)with areas of destructed cells (irregular arrow) ,the surrounded theca cells (T) are disorganized and separated from each other .Toluidine blue X1000.

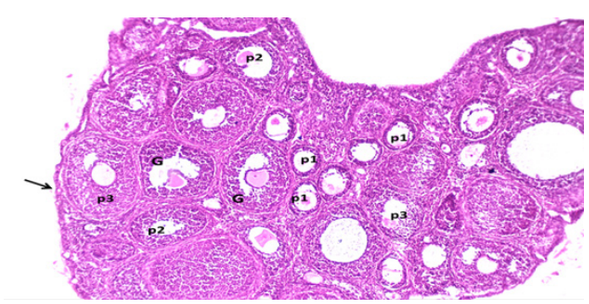


Fig. 11: photomicrograph section in the Ovary of 3week lead and nigella sativatreated rats showing intact surface epithelium (arrow), primary follicles (p1) and secondary follicles (p2) still with destructed nuclei, multiple tertiary follicles (p3) and Graafianfollicles (G) appear with normal shape with intact zonapellucida. H & E X100.

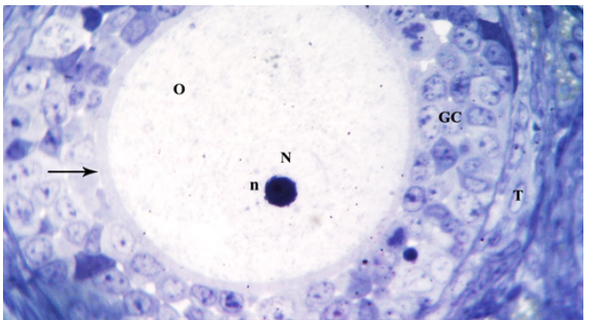


Fig. 12: A photomicrograph of semi-thin section in the ovary of 3weeks lead and nigella sativarat showing an oocyte (O)of secondary follicle with eccentric nucleus (N) has central nucleolus(n) surrounded by regular zonapellucida (long arrow) which encircled by granulosa cells (GC) which surrounded by normal tightly adherent theca cell (T). Toluidine blue X 1000.

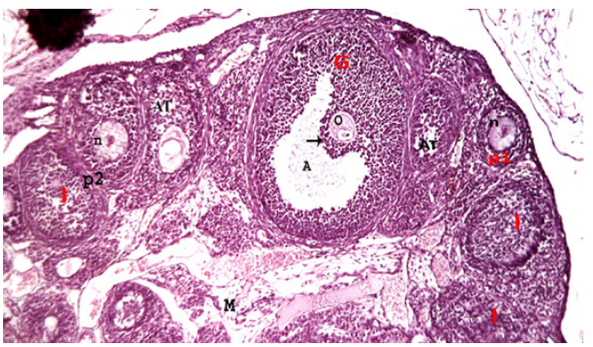


Fig. 13: photomicrograph section in the ovary of 6 weeks control rats showing number of follicles present mainly within the cortex. medulla (M) appear at the center and contains blood vessels and loose connective tissue; primary follicle (P1) and secondary follicle (p2) surrounded with granulosa cells and central nucleus (n), Graafianfollicle (G) with largeantral space (A) and 2ry oocyte (O) surrounded by corona radiate (arrow), Multipleatretic follicles (AT) and corpus luteum (l) also appear.H & E X100

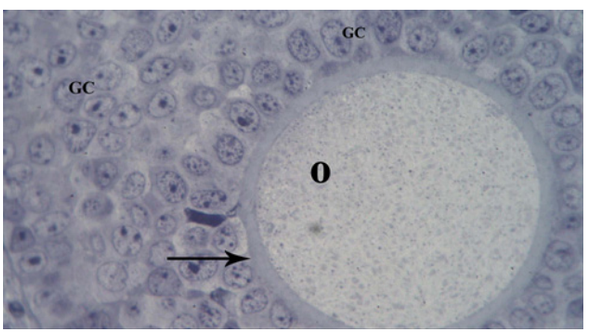


Fig. 14: A photomicrograph of semi-thin section in the ovary of 6weeks control rat showing an oocyte of secondary follicle(O) rich in cytoplasmic material surrounded by zonapellucida (long arrow) which encircled by normal rounded granulosa cell (GC) that form corona radiate around oocyte.Toluidine blue X 1000.

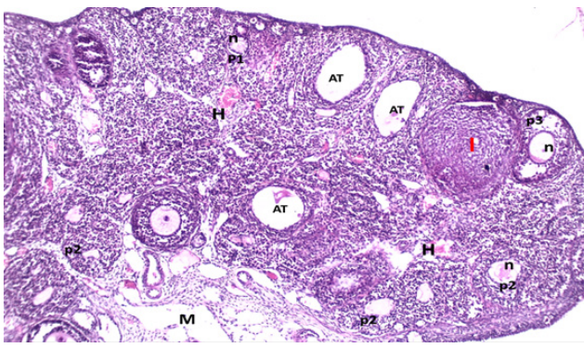


Fig. 15: photomicrograph section in the ovary of 6 weeks lead treated rats showing number of follicles present mainly within the cortex and medulla (M); primary follicle(P1), secondary follicle (p2) andtertiary follicles (p3) appear with damage granulosa cells and shrunken nucleus (n); multipleatretic follicles (AT) and areas of hemorrhage (H) appear in-between the follicles, corpus luteum (l) also appear.H & E X100.

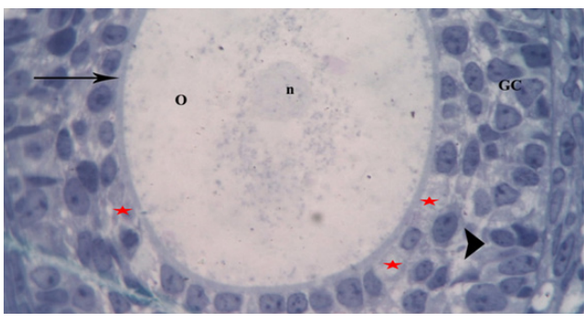


Fig. 16: A photomicrograph of semi-thin section in the ovary of 6weeks lead treated rat showing part of an oocyte (o) of secondary follicle contains faint nucleus (n) and destructed cytoplasm surrounded with a thin zonapellucida (long arrow), and irregular granulosa cells (GC) with dark stained nuclei (head arrow), corona radiate shows multiple empty spaces (stars). Toluidine blueX1000.

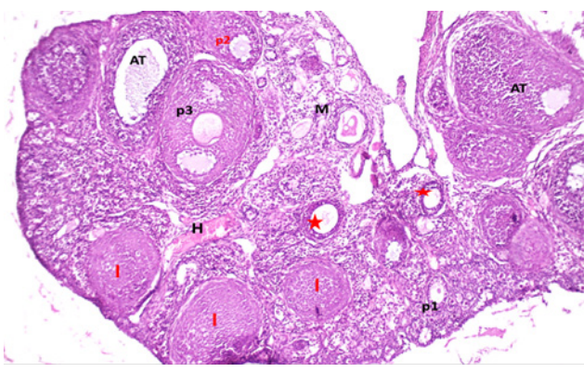


Fig. 17: photomicrograph section in the ovary of 6 weeks lead and nigella sativatreated rats showingmultiple types of follicles present mainly within the cortex and medulla (M) containing blood vessels and areas of hemorrhage (H), normal primary follicles (p1) appear but still others appear destructed (stars), secondary follicle (p2) showing destructed oocyte but the granulosa cells appears normal, tertiary follicle (p3) appears normal,multiple corpus luteum (l) andatretic (AT) folliclesalso present. H & E X 100

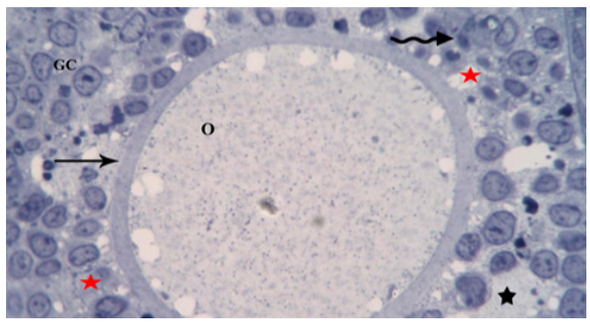


Fig. 18: A photomicrograph of semi-thin section in the ovary of 6 weeks lead and nigella sativatreated rat showing part of an oocyte of secondary follicle (o) which appear normal except for some vacuolation with normal surrounding zonapellucida (long arrow),granulosa cells appearnormal(GC) but some cells appear shrunken(irregular arrow),empty spaces still present in the corona radiate (red stars)and in-between granulosa cells(black star) .Toluidine blue X1000.

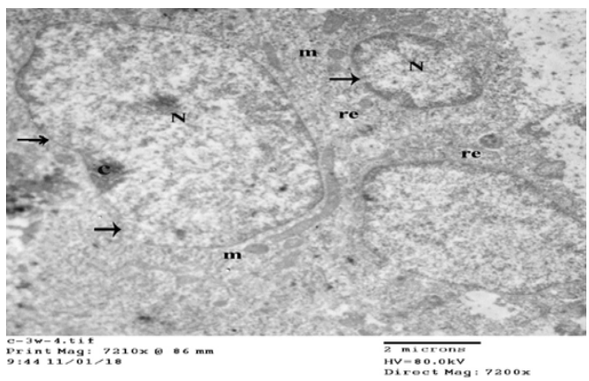


Fig. 19: An electron micrograph of the ovary of 3 weeks control rat showing multiplegranulosa cells containing large nuclei (N) surrounded by intact nuclear membrane (arrows) with condensed chromatin material (c). Their cytoplasm contains intact mitochondria (m) and rough endoplasmic reticulum (re).X 7200.

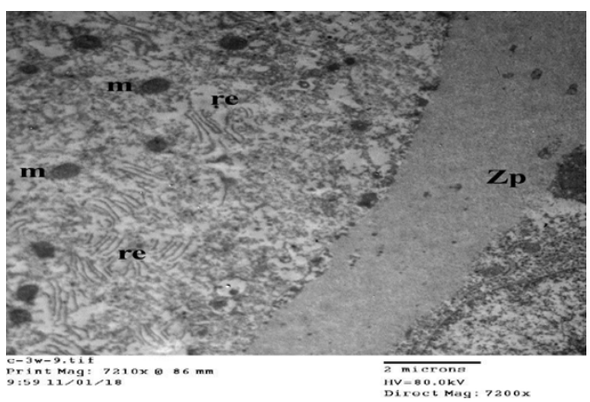


Fig. 20: An electron micrograph of the ovary of 3 weekscontrol rat showing oocyte of secondary follicle with smooth regular zonapellucida (zp), the cytoplasm of the oocyte shows abundant well organized cell organelles; mitochondria (m) and ribosomes (re). X 7200.

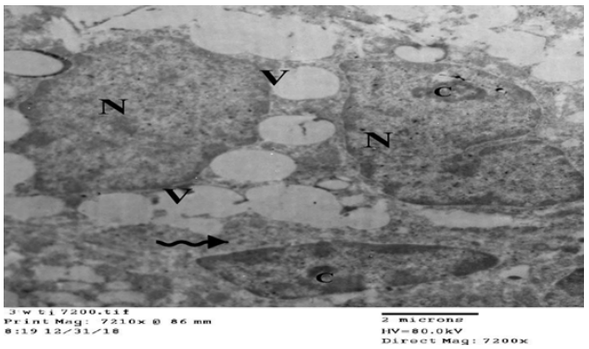


Fig. 21: An electron micrograph of the ovary of 3weeks lead treated rat showing multiple shrunken granulosa cells with destructed cytoplasm and loss of its organelles (irregular arrow), darkly stained shrunken nuclei (N) appear with condensed chromatin (c), multiple vacuoles (V) appear in-between granulosa cell.X 7200.

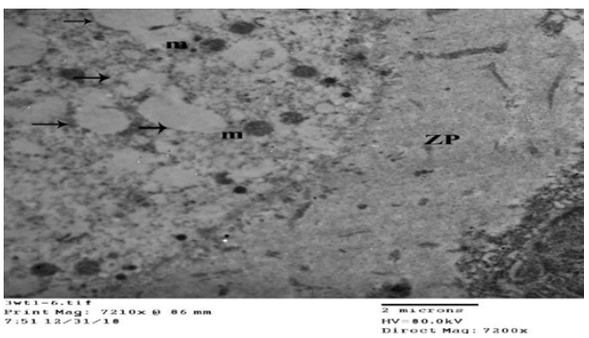


Fig. 22: An electron micrograph of the ovary of 3weeks lead treated rat showing part of the oocyte of secondary follicle withirregular outline of the zonapellucida (zp), multiple vacuoles appeared in the cytoplasm of the oocyte (arrows), loss of ribosomes and reduction in the size and darkness of the mitochondria (m).X 7200.

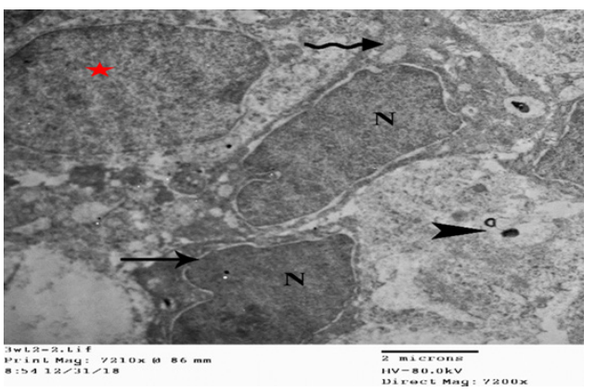


Fig. 23: An electron micrograph of the ovary of 3weeks lead and nigella sativa treated rat showing multiple granulosa cells; one has normal nucleus (*), other granulosa cells(long arrow) appear with darkly stained shrunken nucleus (N) and loss of surrounded organelles and vacuolated cytoplasm (irregular arrow), empty area also appears. (Head arrow). X7200.

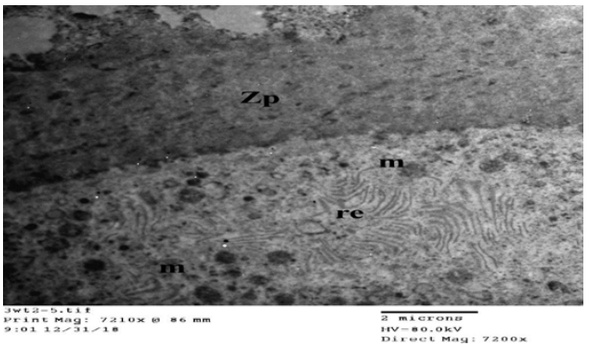


Fig. 24: An electron micrograph of the ovary of 3 weeks of lead and nigella sativatreated rat showing part of the oocyte of secondary follicle surrounded by intact regular zonapellucida (zp), the cytoplasm of the oocyte appear with preserved organelles; multiple mitochondria(m) and ribosomes(re).X 7200.

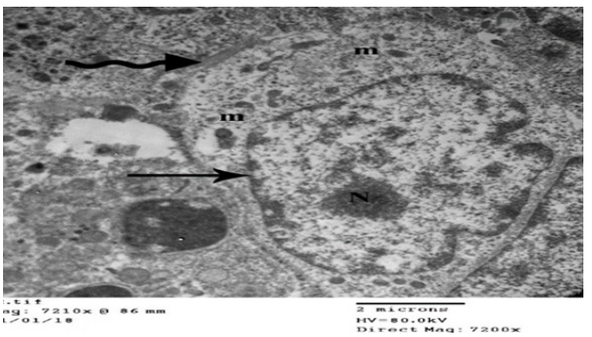


Fig. 25: An electron micrograph of the ovary of 6weeks control rat showing large granulosa cell surrounded by intact well defined cell membrane (irregular arrow) with large nucleus has an apparent nucleolus (N) surrounded by intact well defined nuclear membrane (long arrow). The cytoplasm contains multiple mitochondria (m) and free ribosomes. X7200.

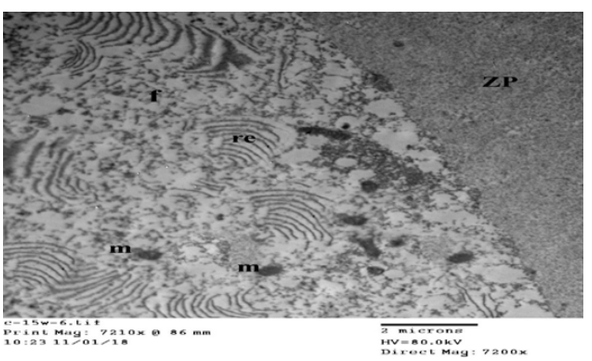


Fig. 26: An electron micrograph of the ovary of 6weeks control rat showing oocyte of tertiary follicle surrounded by smooth regular zonapellucida (zp), the cytoplasm of the oocyte shows well organized organelles; mitochondria(m), free ribosomes (f) and rough endoplasmic retinaculum(re).X 7200.

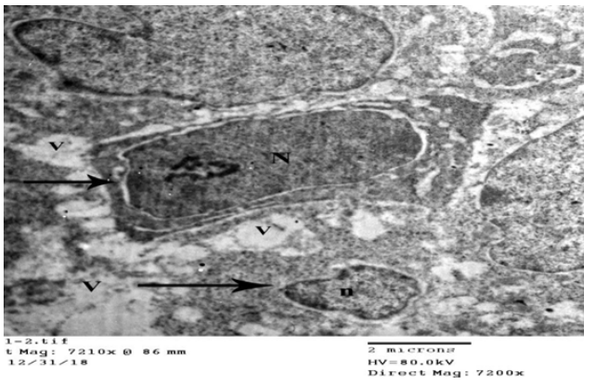


Fig. 27: An electron micrograph of the ovary of 6weeks lead treated rat showing multiple granulosa cells with different sizes (long arrow), one with darkly stained nucleus (N) and another with shrunken nucleus (n) and loss of surrounded organelle, there are multiple vacuoles (V) appear in-between the granulosa cells.X7200

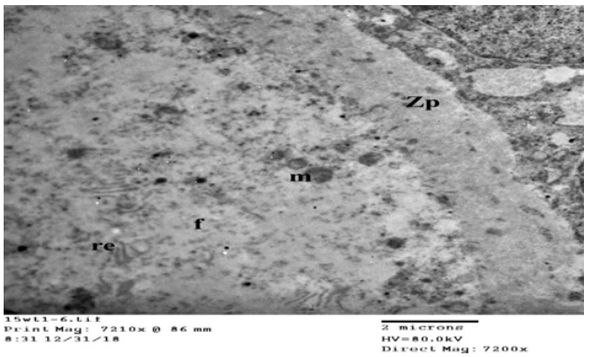


Fig. 28: An electron micrograph of the ovary of 6 weeks lead treated rat showing part of an oocyte surrounded by thin, irregularzonapellucida (zp), the oocyte cytoplasm showing destructed organelles; shrunken mitochondria (m), few destructed rough endoplasmic reticulum (re), scanty free ribosomes (f).X 7200.

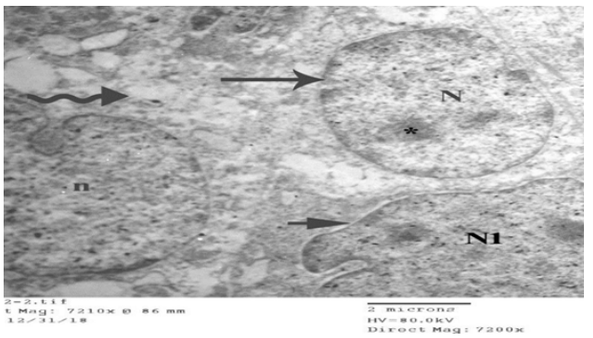


Fig. 29: An electron micrograph of the ovary of 6weeks lead and nigella sativatreated rat showing different granulosa cells; one (long arrow) restore normal appearance with normal nucleus (N)and condensed chromatin (*) but the cytoplasm still losses its organelles,anothergranulosa cell appear with disturbed chromatin of itsnucleus(n), other (short arrow) has normal nucleus (N1) and cytoplasm, some vacuoles still appears in-between the cells(irregular arrow). X7200.

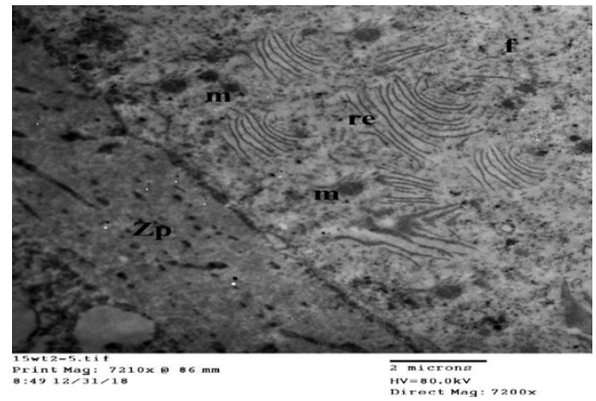
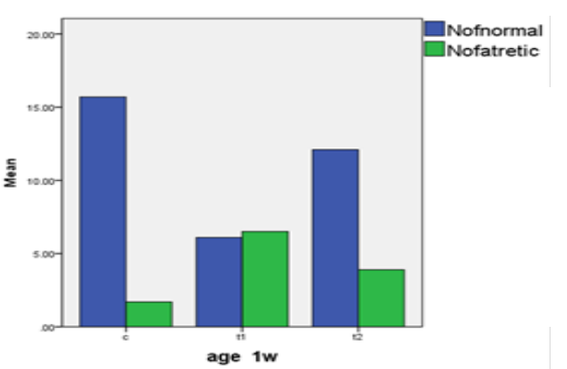


Fig. 30: An electron micrograph of the ovary of 6weeks lead and nigella sativatreated rat showing part of the nucleus surrounded by regular zona pellucida (zp), the cytoplasm of the oocyte showed preserved organelles; mitochondria(m), intact rough endoplasmic retinaculum(re) and free ribosomes(f).X7200.

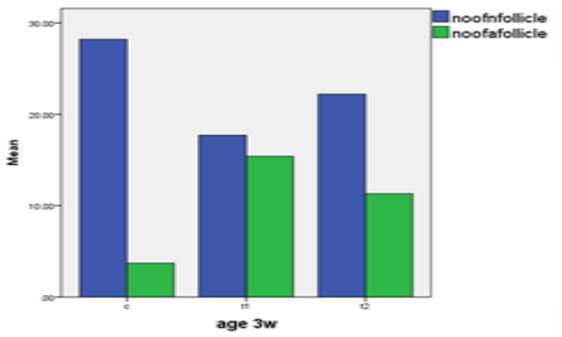
Table1: Showing the mean value± standard deviation of the number of normal and atretic follicles in both control and treated groups.

	NO of normal follicles(N follicles)	No of atretic follicles(A follicles)
1 week		
Control	15.7±1.8	1.7 ± .82
Lead treated (t1)	6.1±2.5***	6.5±1.96***
Lead and nigellatreated (t2)	12.1±1.6***	3.9±1.66***
3 weeks		
Control	28.2±2.74	3.7±.67
Lead treated (t1)	17.7±2.21***	15.4±2.17***
Lead and nigellatreated (t2)	22.2±2.35***	11.3±1.89***
6weeks		
Control	11.6±1.26	4.3±.67
Lead treated (t1)	7.9±1.14***	8.1±1.3***
Lead and nigella (t2)	10±1.12**	5.3±.87**

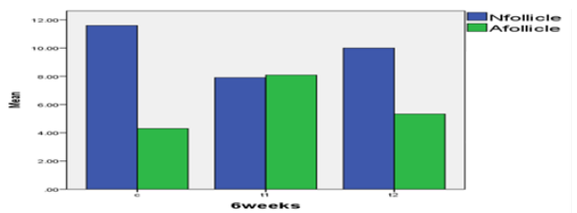
$P \leq 0.01$ (**) → High significant difference, $P \leq 0.001$ (***) → Very high significant difference.



Histogram 1: Mean number of the normal and the atretic follicles of control and treated groups at one week age.



Histogram 2: Mean number of the normal and the atretic follicles of control and treated groups at 3 weeks age.



Histogram 2: Mean number of the normal and the atretic follicles of control and treated groups at 6 weeks age.

DISSCSSION

The light microscopic examination of lead treated animals showed that there was damage in primordial germ cells and primordial follicles; also there were changes in the ovarian architecture with the appearance of cavities among the stromal cells and destruction in the granulosa cells surrounding the follicles.

These results were in acceptance with previous study[19] who observed that prenatal lead exposure can directly cause ovarian failure by extensive follicular destruction which resulted mainly from loss of ovarian steroid hormones and disruption of neuroendocrine feedback leading to increased levels of FSH and LH, also their reproductive toxicants caused direct damage to ovarian follicles and eventual ovarian failure.

Previous studies also support the present results and showed that lead exposure cause edema and necrosis of the ovarian follicles[20] and dysfunction of folliculogenesis with an increase in the number of atretic follicles[21] as it may significantly alter steroid production and gonadotrophin binding in the ovaries of adult rats which lead to atresia of ovarian follicles[22].

On the same line Dhir and Dhand showed that administration of ovaries to lead acetate caused structural changes in the form of diffuse edema, necrosis in the ovarian follicles, optical empty spaces, denudation of the ovarian follicles, and different stages of follicle evolution;this may be due to its hormonal effects[23].

Also several studies confirm the harmful effects of lead; lead intoxicated the mice ovarian follicular cells and the oocyte with increase in the destruction of the granulosa cells and atresia of the follicles[24]; the follicles were undergoing degenerative changes and they had lost their normal shape and the arrangement of granulosa cells with pyknosis of the nucleus and dissolution of cytoplasm after lead administration, this may occur because of its disruption to the prooxidant/ antioxidant balance that occurs within mammalian cells[25].

Morphometric results of the present work observed that the number of primary, secondary, tertiary follicles in lead treated animals were decreased compare to controls and with observable increase in the number of atretic follicles.

At the same line Bires *et al*.,[26] noticed that after exposure to lead there was histological changes in the number of ovarian follicles, increase in primary atretic follicles and alterations in the organelles of oocytes, the study explained that this may be due to reduction in LHand FSHbinding sites, which altered steroid production in *vitro* and exerts a direct influence on granulosa cells function.

Previous studies about prenatal lead exposure showed that neonatal lead treat¬ment reduced the number of primary, secondary and antral follicles through inhibition of transition from the primordial to primary follicular stage[27], exposure to lead has effects on the histomorphology of ovary through decrease folliculogenesis[28] also after oral administration of high doses of lead, there was reduction in numbers of ovarian follicles and an increased number of atretic follicles[29].

In the present study electron microscopic results in lead treated animals revealed that lead caused shrunken in granulosa cells, the zonapellucida surrounded oocyte was reduced in thickening, the cytoplasm of oocyte showed multiple vacuolesandscanty nuclear organelles.

This agree with previous results[30] which reported that signs of lead follicular atresia was that granulosa cell were shrunken with irregular cell membrane and loss of organelles, they also noticed that the oocyte organelles became more randomly distributed throughout the cytoplasm, and mitochondria stained more darkly and begun to lose their cristae.

These harmful results may be explained as the heavy metals (lead) induce DNA fragmentation where one of the mechanisms of DNA fragmentation is apoptosis to the cells, in addition it interferes with DNA repair by producing reactive oxygen species (ROS) which cause damage to DNA[31].

Another explanation was that Lead can produce damage to the granulosa cells of antral follicles by increased reactive oxygen species (ROS) that produced oxidative stress which destroy DNA and then stimulate apoptosis in granulosa cells; also lead caused direct apoptosis to the mitochondria through increase intracellular metabolism which is able to stimulate mitochondrial depolarization[32].

Results of present lead and Nigella sativa treated group showed that with further developmental ages nigella sativa reduced damaging effect of lead on the ovarian follicles as there was a mild degree of destruction in granulosa cell and oocyte, some follicles preserved its normal appearance with rounded normal oocyte and granular cells.

This was confirmed by previous studies which showed that administrations of Nigella Sativa against lead acetate prevent alterations in reproductive hormones, sperm parameters and gonadal histology caused by lead acetate in rats[33], also administration of nigella sativa oil improve physiological parameters and oocyte quality through the decrease in values of pulse rate and glucose and the increase in values of blood cells, packed cell volume, total protein after ovarian transplantation[34].

On the other hand, Yadavand Agarwal,[35] reported that the ovarian follicles of female rats following administration of a high concentration of Nigella sativa showed hypertrophy of the theca folliculi, complete destruction of the basement membrane separating the theca folliculi from the zonagranulosa. Degenerative and atrophic changes were observed in the developing oocyte as it caused estrogen inhibition due to its antiestrogen nature.

In the present work the number of normal follicle and atretic follicles in lead and Nigella sativa treated group showed mild difference in comparison with to control group.

This comes in agree with previous results[36] which reported that adult rats exposed to lead acetate had a significant reduction on ovarian function and damage to ovarian follicles while treatment with Nigella sativa caused significant enhancement on the reproductive function with improvement in the number and diameter of Graafian follicles in comparison to the normal structure ,they explained this that Nigella sativa elevated FSH hormone and increasing its receptors on granulosa cells surface.

In the present electron microscopic results after administration of Nigella Sativa, the granulosa cells showed darkly stained nucleus with loss of cytoplasmic organelles while some granulosa cell appeared with normal nucleus, the oocyte nucleus appeared normal with preserved cytoplasmic organelles and surrounded with normal zonapellucida.

Previous results of Alenzi *et al*.,[37] confirm the protective effect of NSO agonist chemicals and showed that combination of nigella sativa oil (NSO) with cyclophosphamide seemed to induced significant protection on the fine structure of follicles and increase the survival rates of normal follicles than cyclophosphamide-treated group, they suggested that the antioxidative properties of NS may have protected the follicular cells from cyclophosphamide induced destruction.

At the same line administration of nigella sativa oil improved the ultrastructural changes in the testicular component produced by aluminum chloride and explained this that nigella sativa decreased oxidative stress produced by aluminum chloride and protection of the antioxidant enzymes of the testis this promoting cellular growth and metabolism[38].

Also Azzawi and Baraaj showed that the Normal dose of nigella sativa treatment produce significant protection to the renal tissue against injury induced by rifampicin in rats and explained this that the protective effect of N. sativa in Rifampicin- induced injury might be due to prevention of lipid peroxidation as well as free radical properties of its active component[39].

CONCLUSION

- Exposure of pregnant and lactating mothers to lead acetateaffect the ovarian follicles in the form of delayed development of primordial follicles, damage to granulosa cell and degeneration to the oocyte.
- Adding of natural product as nigella sativa can decrease the hazards effect of lead acetate on the ovary.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير خلات الرصاص على نمو المبيض فى فترتى ما قبل البلوغ والبلوغ في نسل الجرذان البيضاء والدور الوقائي المحتمل لزيت الحبة السوداء

سلوى محمد عويس[٢]، صلاح رشدي احمد[١]، اسماء صبري باسط[٢]

[١]قسم التوليد وامراض النساء، [٢]قسم التشريح الأدمي وعلم الاجنة، كلية الطب - جامعة سوهاج - مصر

مقدمة: هناك استجابات مختلفة للتعرض للرصاص بما في ذلك انخفاض الخصوبة ، والإجهاض التلقائي، وانخفاض الوزن عند الولادة، وضعف في تكوين الأجربة، وحتى تلف المبيضين. أكدت عدة تقارير فائدة زيت الحبة السوداء بسبب احتوائها على أكثر من مائة مكون كزيت متطاير وفيتامينات وعناصر نادرة. في الآونة الأخيرة، كشفت الدراسات السريرية والحيوانية أن مستخلص الحبة السوداء له العديد من التأثيرات العلاجية.

هدف العمل: دراسة تطور المبيض لنسل إناث الفئران البيضاء المعرضة لاستات الرصاص أثناء الحمل والرضاعة والدور الوقائي المحتمل لزيت حبة البركة.

المواد والطرق: تم إجراء هذه الدراسة على ثلاث مجموعات مجموعة "أ": تضم ١٥ نسلًا من ٢٠ أمًا ضابطة، المجموعة "ب": تشمل ١٥ نسلًا من ٢٠ أمًا تم علاجهم بأسيتات الرصاص عن طريق الفم بجرعة ٦٤٠ مجم / كجم، المجموعة ج: تتضمن ١٥ نسلًا لـ ٢٠ أمًا بالإضافة إلى نفس جرعة أسيتات الرصاص لكل فأر تم تناول زيت حبة البركة عن طريق الفم بجرعة ١٠ مجم / كجم.

النتائج: تسبب التعرض للرصاص قبل الولادة في حدوث تغيرات مرضية حادة في حويصلات المبيض في شكل تأخر في نمو الحويصلات البدائية وتلف في الخلايا الحبيبية وضمور البويضة. يمكن أن يقلل إعطاء زيت حبة البركة من تأثير أسيتات الرصاص ولكن لا يمنعه.

الخلاصة: تعرض الأم لمنتجات رصاص الأسيتات يسبب آثارًا ضارة على مبايض النسل، يمكن أن يحسن زيت حبة البركة من هذه الآثار الضارة.