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RESEARCH ARTICLE

STUDY THE ADVERSE EFFECTS OF EXPOSURE TO LEAD ACETATE ON MICE OVARIAN TISSUE.

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Abstract

There has been growing concern about human reproductive perturbation by xenobiotics encompass drugs, occupational, and environmental exposures of toxicant. Lead considered as one that could affect the gonadal structure and functions. Exposure to lead is still a major medical dilemma in both environmental and occupational settings. The toxicity of lead, on adult female reproduction results in reduce fertility, less ability to sustain pregnancy, and low pregnancy outcomes. As there is an increase usage of electrical generators that depend on lead-based gasoline by Iraqi people which lead to increase the air pollution with this toxic substance induced me to detect through this study its possible negative effects on the ovarian tissue using the mouse as a model. Albino mice of 8-10 weeks age weighing between 25-30 grams divided randomly into two groups of 16 each. The first group (control group) (c), was not given lead acetate whereas group II were given lead acetate in dose 0.3 mg /kg BW. / Day, intraperitoneally, for 14 days. Animals of each group scarified to get their ovaries which removed by dissection, processed with the standard histopathological technique for the quantitative assessment of follicle numbers and to study the histological changes in the ovarian tissue. The result of this study revealed that low dose of lead acetate injected to mature female mice intraperitoneally for 14 days cause a non-significant decrease in the number of follicles. But ovaries belong to experimental group revealed histopathological alterations in the various components of their ovaries. This study designed to detect the possible deleterious effect of low dose of lead acetate on the ovarian tissue of mature female mice.

Introduction:-

Environmental pollutions caused by industrial and domestic wastes are nowadays the greatest concern in public health [1,2] and the humans are exposed to various types of environmental contaminants at different stages of their life span, majority of them are harmful [3]. In fact, a number of heavy metals are still widely used in industry and lead, in particular, generally considered as one of the most toxic metals to humans as well as animals [4]. Lead toxicity is well known to humanity since ancient times and mentioned in documents left by the Greeks, Romans, Arabs, and Egyptians [5]. This heavy metal still mined and added to many products including paints, eye cosmetics, gasoline, and water pipes. It considered as one of the most hazards that affect all biological systems through

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exposure from air, water and food sources [6]. Clinical manifestations of lead toxicity including the central and peripheral nervous systems, renal and gastrointestinal systems were improved [7]. Significant decrease in red blood cells count, hematocrit (Hct) and hemoglobin (Hb) seen in rats and human with high blood lead levels [8, 9]. Moreover, brain damage, mental impairment with severe behavioral problems, as well as anemia, neuromuscular weakness and coma [10] are clinical manifestations of lead toxicity.

Lead considered as one of the reproductive toxicant [11]. As previous studies have shown that chronic exposure to lead has deleterious effects on both male and female reproductive system. It causes a significant decrease in the weights of the testis, epididymis, prostate and seminal vesicles in male rats with high blood lead level [12]. While in females, chronic exposure to lead causes disorders in the hormonal function that affecting the ovary and reduced fertility [13]. Qureshi et al., [14] improved that lead affects female fertility, is the classical sings of lead poisoning in pregnant females. Mogra *et al.,* [15], also noted that prenatal exposure to lead has toxic effects of human fetus including increased risk of preterm delivery, low birth weight, impaired mental development with adverse effects on development of gonads [16].

A study in the USA involving 140 couples participating in IVF treatment showed that higher lead levels is associated with low fertilization rates [17]. Meanwhile, Tang, N. and Zhu, Z.Q. **2003** [13] cited that, the incidence of polymenorrhea, prolonged and abnormal menstruations, and hyper menorrhea was significantly higher in the group of female workers at mean age of 32 years employed in a storage battery plant and a capacitor factory than in controls. The authors conclude that occupational lead exposure result in the impairment of the functions of reproductive system. Qureshi *et al.,* [14] reported that lead treated females show reduced number of pregnancies, small litter size and decreased body weight. While Goyer, 1990 cited that a few Pb studies conducted on females revealed mostly sterility, miscarriages, chromosomal aberration, premature delivery, and infant mortality in humans and animals from exposure in utero [18]. Furthermore, cases of frequent abortions and abnormal menses have demonstrated in woman working in lead based industries [19]. However, Panwar *et al.,* [20] noted that the ovary has an important role in reproduction, as the development, maturation and ovulation of female gametes occur within the ovarian follicles, which is the functional unit of the ovary. It contains the oocyte that may eventually ovulate, undergo fertilization and form an embryo. It also secrets the steroid and protein hormones required for maintenance of the ovarian cycle, the secondary sex characteristics and preparation of the uterus for implantation [21]. Junaid et al., reported that lead acetate have a significant role in arresting growth and maturation of the ovarian follicles upon oral administration to mice [22]. A previous of a series studies improved the possible links between low level lead exposure and the adverse effects on reproductive system including menstrual status and pregnancy outcome as higher prevalence menstrual disturbance, spontaneous abortion and threatened abortion in exposed females [23], indicates that reproductive toxicants produce their adverse effects in wide variety of ways in lead exposed females [24].

Materials and Methods:-

All experiments were performed on 32 mature female Swiss-Webster mice; their age ranged between (8-10) weeks with a body weight ranged between 25-30gm., obtained from the colony of the animal house of the High Institute of Infertility Diagnosis and Assisted Reproductive Techniques, Al-Nahrain University. These 32 mature female mice divided randomly into two groups of 16 each. The first Group (control group) (c), was not given lead acetate whereas group II, were given lead acetate in dose 0.3 mg /kg BW / day, intraperitoneally for 14 days. Mature female mice were injected with lead acetate 0.3 mg /kg bw/ day, intraperitoneally, and continued for 14 days for experimental groups while the control group were injected normal saline with same volume and frequent as that used in the experimental group. At day 14, 16 animals of each group were scarified; an incision was made in area of the abdomen to remove the ovaries. The abdominal cavity opened, one ovary above the oviduct was grasping firmly with fine forceps. The same performed to the other ovary. The intact ovaries were removed and placed in a dish containing warm normal saline, washed and weighing by sensitive electrical balance, then fixed in Bouin's solution for routine histological techniques; paraffin sections with 5-micron thickness were prepared and stained with hematoxylen eosin stain for histological study [25]. Slides were examined to study the histological changes in the ovarian tissue, and the numbers of different ovarian follicles were recorded. Statistical analysis according to SPSS 7.5 version to determine the mean and standard error of the mean, P-value ≤ 0.05 considered significant in this study [26].

Results:-

Effect of low dose of lead acetate on the number of follicles:-

A non significant decrease in the number of follicles of ovaries of mature female mice injected with lead acetate at dose 0.3 mg /kg bw/ day, intraperitoneally for 14 days was recorded, table (1).

Histological study:-

Histological observations:-

The histological sections of the mature female mice ovary from control group shows a well-defined ovary at various stages of growth, the oocyte enclosed in a healthy antral follicle, it also shows a mature Graafian follicle with ovum and its nucleus and nucleolus, which surrounded by the follicular cells the corona radiata. Zone of granulosa cells well defined with a demarcating basement membrane. The ovum attached to the membrane granulosa by cumulus oophoricus figure (1). While a mature female mice ovary belong to experimental groups observed that almost all follicles (at any stage of development) had a denuded and degradation of oocyte, zona pellucida is not visible, degenerative corona radiata cells, follicular antrum full with fluid and show massive apoptosis, with many vacant areas. The granulosa cells in the region of cavity are reduced in number and scattered with increased incidence of apoptosis figure (2, 3, 4 and 5), The counting of various stages atretic follicle in the treated groups of albino mice showed that as the blood lead levels increased, the percentage number of atretic follicle increased.

Table (1):- Changes in number of follicles in the ovary associated with the administration of 0.3 mg /kg BW/ day, intraperitoneally of lead acetate to mature female mice for 14 days.

	Number of follicles
Control	**5.0±6.0**
Treated- 0.3 mg/k. b.w	**3.6±1.04**

***p<0.05 Significant**

****P<0.01 High significant**

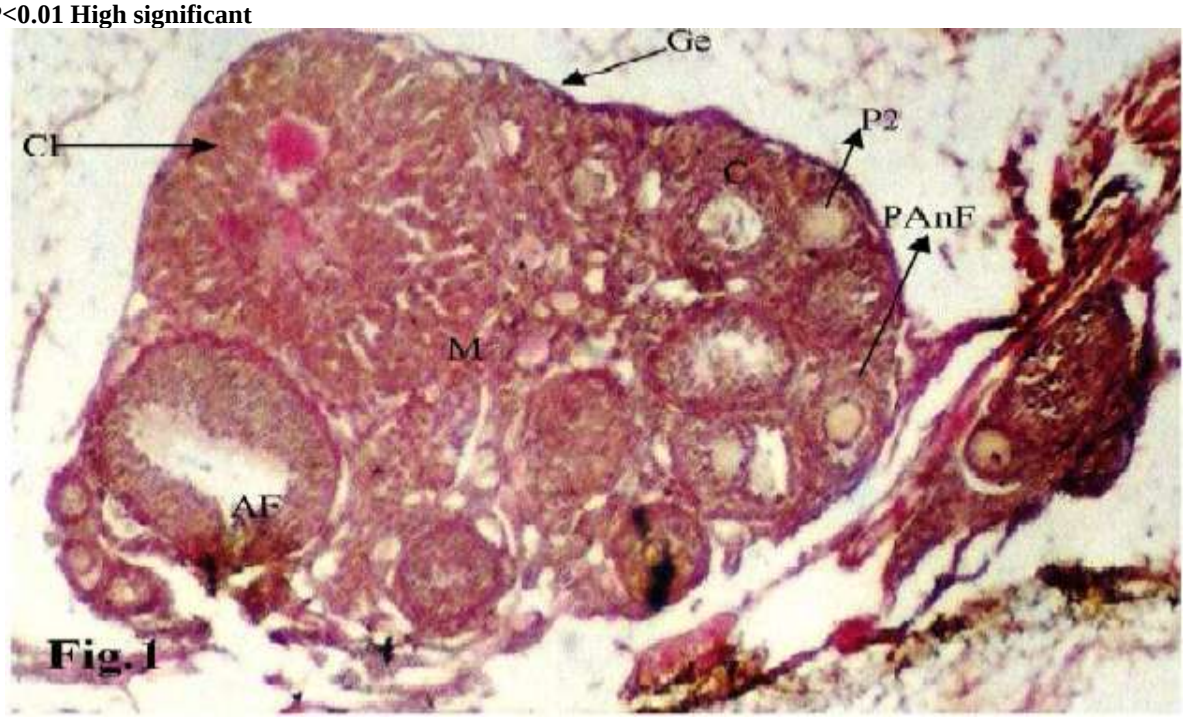

Fig.1:- Ovary of control mice showing normal structure and distribution pattern of various ovary components, germinal epithelium (Ge), cortex (C) and inner medullary region (M). Different types of developing follicles, primary (P2) ,preantral follicles (PAnF) and atretic follicles (AF) are in normal state. This figure also shows the normal structure of corpus luteum (Cl) [24].

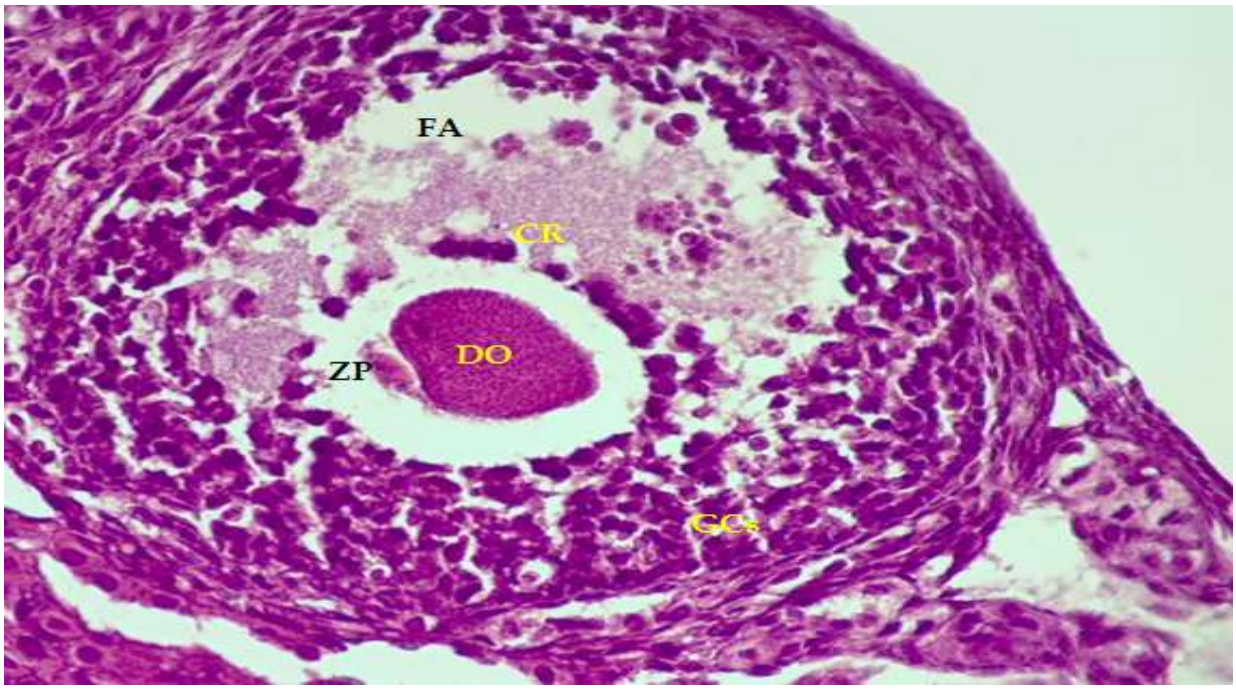

Fig. 2:- Micrograph illustrate the Graafian follicle from a treated ovary shows a denuded oocyte (DO) and zona pellucida is not visible (ZP). The entire region shows degenerative corona radiata cells (CR) and follicular antrum (FA) full with fluid shows massive apoptosis, while the granulosa cells (GCs) in the region of cavity are reduced in number and scattered with increased incidence of apoptosis (400X, H&E).

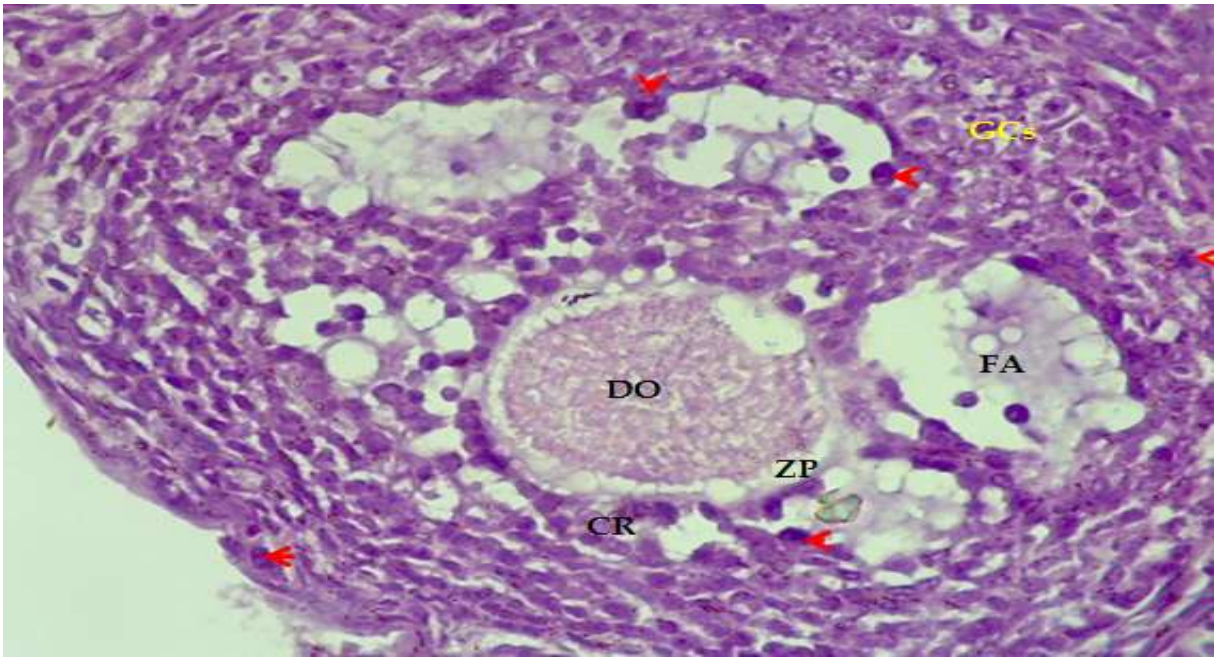

Fig.3:- A histological section of the antral follicle from a treated ovary showing a degenerative oocyte (DO), the zona pellucida (ZP) has not identified. The cells surrounding the ovum show certain cavities. Degenerative corona radiata (CR) clearly observed. Follicular antrum (FA) full with fluid shows many vacant areas. The granulosa cells (GCs) with pyknosis (red head arrows) of the nucleus and dissolution of cytoplasm (400X, H&E).

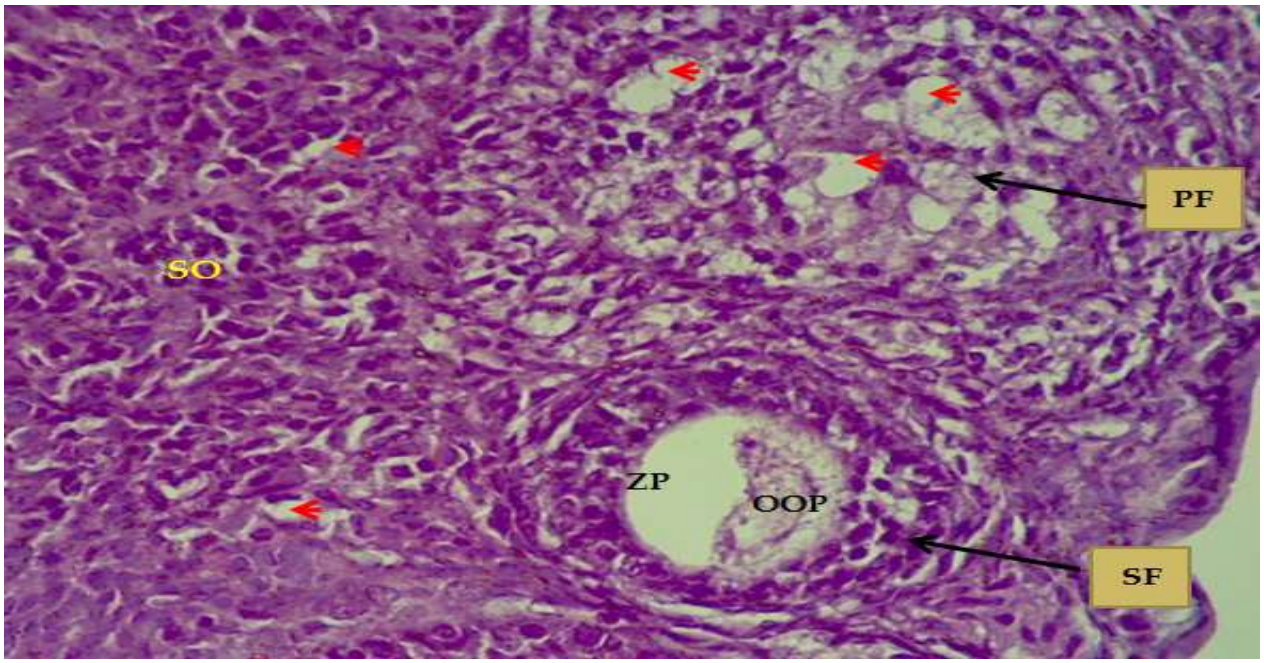

Fig.4:- Micrograph illustrate a histological section of the secondary follicle (SF) from a treated ovary shows degenerative changes in the ovum. There is shrinkage and shift of ooplasm (OOP) to one side with associated non-visibility of nucleus and nucleolus and absence of zona pellucida (ZP). The typical appearance of a primary follicle (PF) has been totally lost. In the stroma of the ovary (SO) and entire of primary follicle (PF) many vacant areas (red arrows) were seen, some of these are full of fluid (400X, H&E).

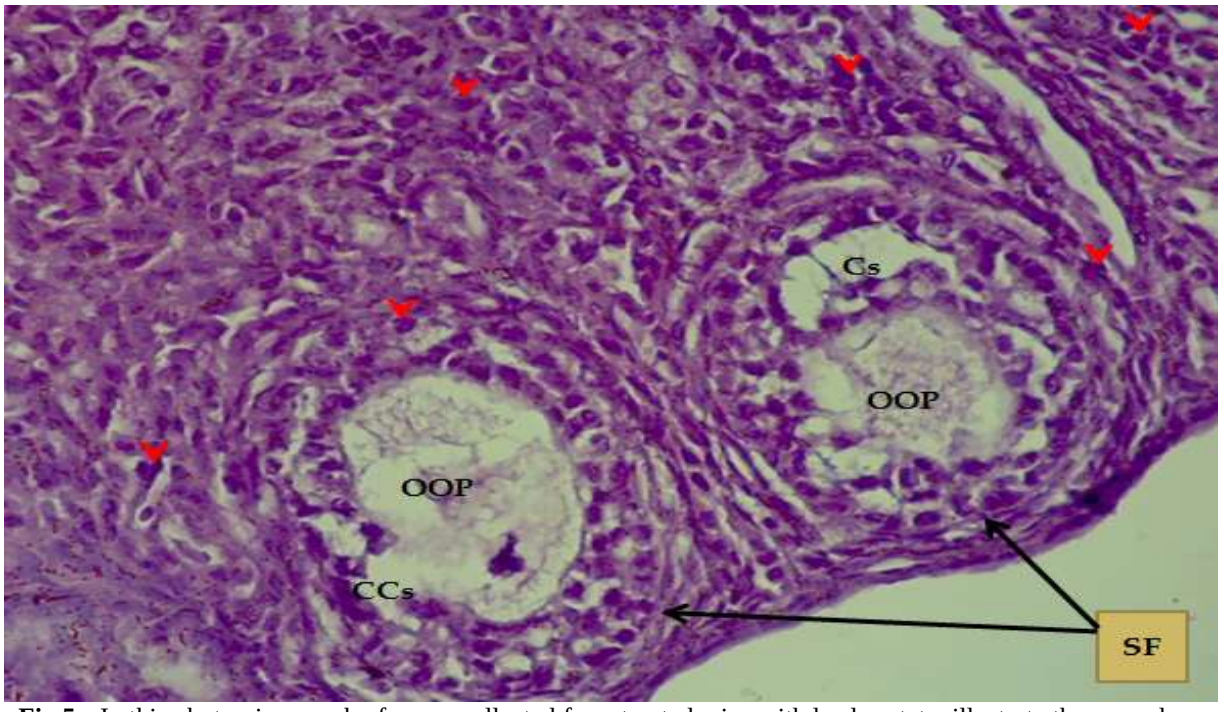

Fig.5:- In this photomicrograph of ovary collected from treated mice with lead acetate, illustrate the secondary follicles (SF), the region of the ovum show degenerative changes. The granulosa cells (GCs) shows pyknosis (red head arrows) of the nucleus and dissolution of cytoplasm. The cells surrounding the region of ovum show cavities (Cs). Ooplasm (OOP) shows marked degenerative changes without any indication of residue of nucleus or nucleolus. Some of the cuboidal cells (CCs) are sloughing (400X, H&E).

Discussion:-

Lead is a prevalent environmental pollutant [27]. Many studies mention that lead result in a direct impairment to the mouse ovaries [22], and illustrate the morphological changes of ovarian toxicity by counting the variable stages of follicular development using different doses of lead acetate [22]. In fact reproductive toxicity is the adverse effects of chemicals on gonadal structure and functions reduce fertility and deteriorate gamete function [28].

The results of this study showed that injection of lead acetate at dose 0.3 mg /kg BW / day, intraperitoneally for 14 days causes a non significant reduction in the number of ovarian follicles. Previous studies proved that the reduction in the number of follicles occur when lead acetate given at high doses. Shaukat and his college's study the oral administration of lead in high doses (2, 4, 8 mg /kg Bw. / day, respectively) could causes a reduction in the number of ovarian follicles [29]. While no significant difference between the mean numbers of ovarian follicles in the 20 mg/L /day dose, group and control group observed at different stages of postnatal development [30].

In this study treated ovaries revealed that the follicles were undergoing degenerative changes and they had lost their normal shape and arrangement of granulosa cells with pyknosis of the nucleus and dissolution of cytoplasm (Figure3). These observations are like the histological study of Azarnia *et al.,* [31] in his study observed changes in the follicular cells and oocyte of chronically lead intoxicated mice, with perturbation of the follicular membrane and increased pyknosis in granulosa cells, which is marked the follicular atresia. In fact, pyknosis and fragmentation of the inner granulosa cells is the first sign noticed during the atresia in a follicle [32]. Furthermore, reduction in the number of granulosa cells and obvious shrinkage in these cells clearly distinguished in all developing follicles [33]. Bires *et al*., [34] also noted histological alterations in the number of ovarian follicles and the increase manifestation of primary atretic follicle indicated changes in the membrane and organelles structures of oocyte and follicular cells of the stratum granulosum. Junaid *et al*., [22] done a study on lead in humans and animals using different doses of lead acetate (0, 2, 4 or 8 mg/kg/day) for 60 day (5 day/wk) by oral gavages and concluded that while small medium follicles were significantly affected even at the lowest dose (2 mg), the large follicles were affected mostly at the highest dose.

In histological sections of this study of treated ovaries the principal observation was degeneration of ova in all the atretic follicles which were in deferent stages of development (Figure 2,3, 4 and 5) is in agreements with the fact that follicular atresia is responsible for the resumption of meiosis and oocyte retrogression in different mammalian species [35].

There is shrinkage and shift of ooplasm to one side with associated non-visibility of nucleus and nucleolus and absence of zona pellucida as the surrounding cells have failed to secrete it (Figure 5). As reported by Takase et al., shrinkage of the ooplasm due to severe loss of granulosa cells by increased apoptosis, followed by detachment of ova from the periphery is supposed to be because of pyknosis and fragmentation of the inner granulosa cells. The detachments were observed from the whole periphery in a circular zone [36]. Ooplasm agglutination makes the ovum physiologically dead. This suggestive of effect of toxins on granulosa cells through blood supply followed by permeation to the ovum causing this agglutination of ooplasm, the sequences of events are very rapid and the animals become infertile immediately [37]. The entire of primary follicle seems to have many vacant areas (Figure 3, 4), it may attributed to that thinning of cumulus oophoricus, freeing of oocyte in to the liquor and finely sloughing of the ovum and entire granulosa at times giving appearance of vacant areas. Low levels of lead could accumulate in the ovaries result in an impediment folliculogenesis [38]. Moreover, daily doses of 50mg/kg of lead acetate injected into the animals induce morphologic alterations in the parenchyma of ovaries and adrenals [39], by binding to steroid hormone receptors, changing the ovarian development and function, through estrogenic, anti-estrogenic, and/or anti-androgenic effects. Lead can have an impact not only directly on LH secretion from the pituitary but also at lower levels of reproductive control because it negatively affects not only LH receptors in the ovary but also estradiol receptors in the body [40]. Roniset *et al*. also improved that lead could perturbation the normal profile of reproductive hormones in animals, at hypothalamic pituitary and at the gonadal levels [41]. Moreover chronic exposure to lead of cynomolgous monkeys resulted in reduced level of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prostaglandins [42] that impede folliculogenesis, with fewer primordial follicles and an increase in atretic antral follicles [43]. Recent studies improved that at least some lead- induced damages may occur because of its penchant for disrupting the prooxidant/ antioxidant balance that occurs within mammalian cells [44]. The mechanism of lead induced oxidative stress involve the effects of lead on membranes, DNA and antioxidant defense systems of the cells [45]. Balasch and Fabregues [46] reported that the control of ovarian stromal cells and germ cell function is a diverse paradigm and oxidative stress may be one of the modulators of

ovarian germ cell and stromal cell physiology. Behrman *et al.*, [47], also noted that reactive oxygen species (ROS) is one of the modulation of physiological reproductive functions such as oocyte maturation and granulosa cells degradation [48]. Agarwal et al., [49] revealed that ROS affect many physiological processes from oocyte maturation to fertilization. Heavy metals have properties that mechanically effect antioxidant processes. In fact lead and other heavy metals have high empathy for glutathione (GSH), which is the primary intracellular antioxidant [50]. Lead-induced depletion of intracellular GSH and elevates levels of malondialdehyde in ovary and other organs suppress the activities of two key enzymes involved in GSH metabolism: GSH synthetase and GSH reductase [51]. However, lead compounds can cause enzyme dysfunction, which possibly their mechanisms of toxicity on the reproductive system [52].

In conclusion Lead exposure even at low level can affect female fertility in human and animals. Lead alters the normal histology and the physiology of the ovary and uterus. It also effects negatively the development of different follicles in the ovary and induces alterations in the estrus cycle. The adenohypophysical-hypothalamic gonadal axis is also affected by lead which plays an important role in controlling many factors responsible for reproduction. This detailed impression of reproductive lead toxicity suggest that reproductive toxicants produce their adverse effects in different ways and multiple sites in lead exposed females and incorporate with all factors induces infertility in affected females.

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