IJPSR (2013), Vol. 4, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 December, 2012; received in revised form, 12 February, 2013; accepted, 22 March, 2013

LEAD TOXICITY AND POSTNATAL DEVELOPMENT OF OVARY

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Keywords:
Lead acetate, Development, Ovary, Mice

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ABSTRACT:

Purpose: The course of human development from conception to adulthood is extremely complex. The developing organism is particularly vulnerable to toxic insult because of rapid cell division and differentiation and severely affected during gestation and lactation. The aim of the present study was to evaluate lead toxicity on the female reproductive system during neonatal period.

Methods: Histopathological alterations in the developing ovaries were examined in neonates from birth to 21st day of weaning on specific days viz. 1, 7, 14 and 21st day of postnatal development. Lead acetate was administered via oral gavaging at 266 mg/kg/bodyweight and 1066 mg/kg/bodyweight to pregnant Swiss mice from 10th day of gestation to 21st day of lactation.

Results: Studies conducted on females revealed mostly miscarriages, premature delivery and infant mortality. Lead suppresses the development of various follicles during fetal and neonatal life.

Conclusion: It appears that lead interferes during specific events of ovarian developmental stages, which may create higher sensitivity for dysfunction in reproductive system during adulthood. The present investigation evaluates the relative influences of prenatal and postnatal exposure of lead acetate on growth and ovarian histology in female offspring during postnatal development.

INTRODUCTION: Metals pose a significant threat to health through occupational and environmental exposure and exert their most serious adverse effects during fetal development. It is well known that lead can pass through the placenta from mother to fetus, accumulates in fetal tissues during gestation [1] and can be obtained through the milk during lactation [2].

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Article can be accessed online on:
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Lead is able to pass through the placenta and breast milk and blood lead levels in mothers and infants are usually similar [3]. A fetus can be poisoned in utero if lead from the mother's bones is subsequently mobilized by the changes in metabolism due to pregnancy and increased calcium intake in pregnancy may help to mitigate this phenomenon [4].

Epidemiological and animal studies have illustrated that trace metals such as lead, cadmium and mercury have the potential to disrupt ovarian function [5]. From high to low doses of lead exposure, there are different responses of lead including reduced fertility, spontaneous abortions, low birth weight, impairment in folliculogenesis, and even damage to

the ovaries are also reported [6]. Animals have shown that low levels of lead accumulation in the ovaries could impede folliculogenesis [7]. A low Pb concentration in the mouse ovary caused dysfunction of folliculogenesis, with fewer primordial follicles and an increase in atretic antral follicles [8].

Oral administration of lead in high doses leads to reduction in the number of ovarian follicles revealing a strong correlation between blood lead level and atresia of ovarian follicles of albino mice [9].

Wiebe *et al.,* [10] found that lead exposure may significantly alter steroid production and gonadotrophin binding in the ovaries of adult rats. The coordinated development of follicles within the ovary is under the control of hormones and growth factors [6].

Lead exposure is practically hazardous to reproduction at certain life stages. In the mouse, those stages are either around the stage of blastocyst implantation [11-13] during which lead cause disturbance in the endocrine interaction between the ovaries and uterus [14, 15] or at the early organogenesis stage on day 8 of gestation [16]. However the effects of inorganic lead compounds on the developing gonads and their germ cells are not clearly known.

The ovary plays a pivotal role in reproduction as the development, maturation and ovulation of female gametes occur within the ovarian follicle. Many studies suggest that lead causes direct damage to the ovaries, resulting in ovarian follicular cysts and fewer corpora lutea at high lead concentrations [17]. Junaid *et al.,* [18] reported that exposure to high lead concentrations caused considerable damage to mouse ovaries.

Bires *et al.,* [19] noted histological changes in the number of ovarian follicles and the increased occurrence of primary atretic follicles indicated alterations in the membrane structures and organelles of oocytes and in the follicular cells of stratum granulosum. Evidence of direct ovarian effects of lead exposure in mice also has been described [8, 18.]

Accumulation of Pb in granulosa cells of the rat ovaries and ovarian granulosa cell toxicities [20] can induce ovarian changes in sheep [19]. Lead can exert a direct influence on granulosa cell function [21].

MATERIALS AND METHODS:

Ethics: The proposed experiments were conducted during 2009-2011 in the Department of Zoology, University College of science, Mohanlal Sukhadia University, Udaipur, and the experimental protocols were approved by the Institutional Animal Ethical Committee of the University NO.CS/Res/07/759.

Test Chemical: Lead acetate: Laboratory reagent lead acetate, manufactured by 'S.D. Fine Chem. Ltd.', Mumbai was used for the experiments.

Animals: Sexually mature Swiss Albino mice with the age of 6-7 weeks, weighing 30 gm were used for the present study. Mice were kept in plastic cages with iron grills and bedded with rice husk in the ratio of 1:4 males and females. Females were examined for vaginal plugs (an indication of presence of sperm in vagina) and separated for the experimental protocol and their gestational days were recorded.

Experimental protocol: The selected pregnant females were divided into three groups; control (distill water), 266 mg/kg/BW (8 mg/animal/day) lead acetate and 1066 mg/kg/BW (32 mg/animal/day) lead acetate groups for females respectively. The animals were kept and maintained under conventional laboratory conditions of temperature and allowed free access to food (standard pallet diet) and drinking water *ad libitum*. Lead acetate was administrated via oral gavaging daily from GD 10 to PND 21. The female pups from each of the groups were randomly selected, sacrificed on postnatal day (PND) 1, 7, 14 and 21st days and their ovaries were taken for histological examinations following routine microtomy and haematoxylin- eosin staining.

RESULTS: The development of both the mammalian oocyte and the somatic cell compartments of the ovarian follicle are highly coordinated; this coordination ensures that the ovulated oocyte is ready to undergo fertilization and subsequent embryogenesis. Our investigation describes the postnatal developmental changes in female reproductive organs of lead induced mice with reference to ovary from birth to lactation period.

Ovarian development from birth to postnatal period:

Control ovary from PND 1 to PND 21: In the time interval between birth and maturity the ovary develops from a uniform fairly simple organ to a

multiform, highly differentiated one. During the infant and juvenile period the ovary is not a dormant organ, but it is the organ in which constant growth, differentiation and degeneration occurs during the whole development. Follicular organization in the ovary of the mouse begins just after birth, and during the infantile period small primary follicles with two layers of typical granulosa cells are developed. At birth, mouse ovaries appear as a solid organ containing only the primordial follicles, some naked oocytes and germ cells and oocytes. At postnatal day 1, the ovary is maintained by surface epithelium enclosing small number of follicles noticed with many dormant follicles and a pool of non-growing oocytes.

The ovary in the newborn mice pup consists of mainly 2 types of cells: 1) oocytes with primordial follicles and 2) the stroma cells. The ovaries in control group contain primordial germ cells and several numbers of primordial follicles in which a single layer of squamous follicular cells is found surrounding the oocyte. It starts differentiating in cortical and medullary region. The stroma consists of stromal cells is concentrated mainly in the center of the organ is not completely distinguished. The ovary shows normal structure of surface epithelium (consisted mostly of a single layer of squamous or cuboidal cells), primordial follicles, partially or completely encapsulated by flattened squamous follicular cells and oocytes having large eccentric nuclei but somewhere only oocytes are seen away from their follicular cells.

The morphological development of the ovary during first week is a slow process. The size of ovary is increased endowed with primordial and primary follicles and established the normal ovarian architecture markedly differentiated from PND 1. The ovary of a seven day old mouse showing stroma mainly concentrated in the centre of the organ and the surface epithelium is clearly seen with clearly defined cortex and inner medullary region. Follicles continue to grow into 3-4 granulosa layers and the second layer of the theca is established however, ovarian architecture is still compact.

The growing oocytes in the early stages of follicle development occupy the inner part of the cortex. The ovary is densely packed with primary follicles of different sizes separated by flattened cells of the stroma and by interstitial cells. The primordial follicles having a single layer of squamous epithelium and a large eccentric nucleus is found in adequate number mainly seen at periphery of the ovary. Smaller follicles are found at cortical region where as larger follicles are visible at central medullary region. Primary follicles are surrounded by a single layer of cuboidal follicular cells, *Zona pellucida* is present between the oocyte and the adjacent follicular cells known as granulosa cells. Along with primary follicles, central or medullary region also consist of some secondary follicles characterized by two rows of granulosa cells with healthy oocyte and distinct nucleus in which nucleolus is also apparent. The developing theca cells and blood vessels can be seen in the medullary region.

Within second week (PND 14) the ovarian development have reached further stages of development as secondary follicle stage, where the oocyte is surrounded by two layers of granulosa cells. On day 14, the ovaries contained little intervening stromal tissue between the numerous follicles with three to four granulosa cell layers and a distinct theca encloses only the granulosa cells. Mouse neonate at PND7 contains only primordial to secondary stage of follicular development whereas day 14 ovary arriving at advance stages of development showing normal histological structure of surface epithelium, cortex and medulla with many developing follicles including primordial, primary, secondary, preantral follicles.

The stroma composed of underdeveloped theca layers and many blood vessels are visible surrounding often individual follicles and diverge towards the periphery. Numerous primary follicles surrounding a single layer of cuboidal follicular cells are apparent. Secondary follicles are also found in adequate number enclosing a single oocyte with two layers of granulosa cells and distinct, homogenous, deeply stained *Zona pellucida* is found between the oocyte and adjacent follicular cells is very clear. In second week the ovary also shows preantral and antral follicle with a fluid filled cavity present among stratum granulosa (3 to 4 layers of follicular cells).

In these follicles, antrum and developing cumulus oophorus along with well-established nucleus is very clear. Atretic follicles (degenerative follicles) are also seen in their normal range. The transfer of an early stage of oocyte development to more advanced follicular stage is not feasible. The following 21 days reveals the later phases of development of the

primary follicles takes on a very distinctive appearance, which differs markedly from the ovary in infancy. Follicle development has progressively established so that simultaneously small, medium and large follicles are very clearly seen and in proper architecture. The characteristic morphological change in the center of the ovary is the conversion of a solid organ to a fine center which is formed by an intricate vascular system. This development of ovarian tissue begins in the third week of life leads to a fully developed central capillary system in adults. The central part of the juvenile ovary looks empty as compared with the central region (medulla) of younger animals which contains many blood capillaries of irregular shapes and dilatations.

There is marked differentiation between medullary and cortex region. The normal structure of surface epithelium and well developed stroma with cortical and medullary regions are clearly visible, since this is the time where follicle development is progressed so that simultaneously small, medium and large follicles with fully developed central capillary system are seen. Primary follicles are present along with cuboidal granulosa cells arranged in a single layer surrounding the oocyte.

Numerous secondary and tertiary follicles are found along with zona pellucida, oocytes showing proper nucleus with darkly stained nucleolus and multiple layers of granulosa cells are also visible. Preantral follicle (distinguished by the presence of more than two layers of granulosa cells, with the follicle having not fully developed antrum) and antral follicles (the larger ones that had a fluid-filled cavity known as the antrum) are also apparent with a fluid filled cavity antrum present among the granulosa cells in its original number, shape and position mainly distributed in the core of the ovary.

Thecal cells are present in normal state and dispersed throughout various follicles. Atretic follicles are in normal range having degenerated granulosa cells and showing dissolution in cytoplasm (**Plate 1**).

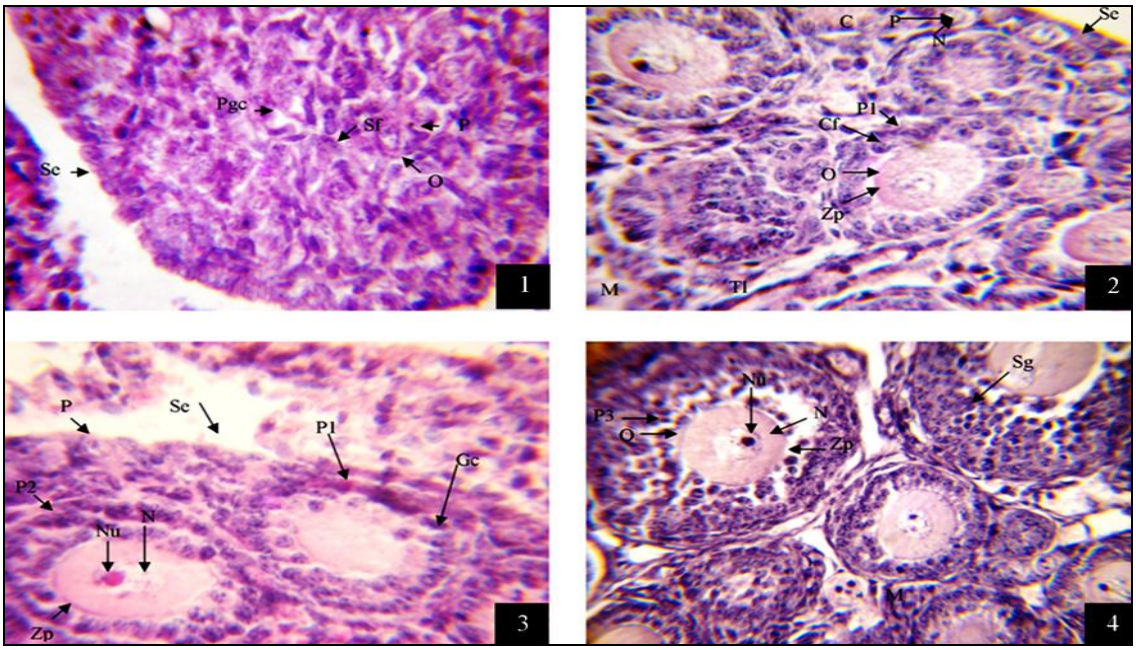

PLATE 1: T.S. OF OVARY OF CONTROL MICE SHOWING POSTNATAL DEVELOPMENT DURING DIFFERENT POSTNATAL DAYS (HAEMATOXYLIN & EOSIN STAIN 450X)

Fig. 1: Ovary of control animals on PND 1 showing normal distribution patterns of various ovary components. Surface epithelium (Se) enclosing differentianting primordial follicle (P) and squamous follicular cells (Sf) surrounding oocyte (O) is in normal condition. Primordial germ cell (PGC) can also identified.

Fig. 2: Control ovary showing normal structure of surface epithelium (Se), Cortex (C) and inner medullary (M) region on PND 7. Primordial follicles (P) with large eccentric nucleus (N) mainly found at periphery. Primary follicles (P1) are visible having single layer of cuboidal follicular cells (Cf) and oocyte (O) with zona pellucida (Zp). Theca cells can also be identified (Tl).

Fig. 3: Control ovary showing normal structure of ovary on PND 14. Different types of developing follicles i.e. primordial (P), primary (P1) and secondary (P2) are with normal structure. In secondary follicles (P2), granulosa cells (Gc) and zona pellucida (Zp) is visible.

Fig. 4: Control group ovary showing characteristic medullary region (M) on PND 21. Different types of developing follicles with zona pellucida (Zp) and oocyte (O) showing proper nucleus (N) and multiple layers of granulosa cells (Gc). Tertiary follicle (P3) showing normal structure of stratum granulosa (Sg) and oocyte (O) is visible with distinct nucleus (N) and nucleolus (Nu).

Lead treated ovary from PND 1 to PND 21: Treatment of lead in mother caused deviation from the normal developmental pattern in mouse on postnatal day 1. Changes in ovarian architecture reveal the appearance of cavities among the stromal cells cause disturbance in their regular organization. The synchronized development of ovarian tissue is perturbed by inducing alteration in normal histoarchitecture by lead acetate. In lower dose (8mg) the shape of the ovary became irregular and structure of germ cells and surface epithelium is altered.

The normal proliferation of oocyte and germ cell is more disturbed in higher dose (32mg), the ovary appears slightly different as primordial follicles are assembled in few groups intercalated in small part of stroma.

The structure of primordial follicle is slightly deviated from control in PND7 ovary, in lower dose (8mg) squamous epithelium is not normal and the position of oocyte is disturbed. Primary follicles are compact, compressed, shows disorder in arrangement of granulosa cells, oocytes are also not in normal shape and reduced in size, exhibits dissolution in cytoplasm and rarely seen with nucleolus. Zona pellucida surrounding the oocyte is damaged in most of the follicles, normal development of theca is disturbed and granulosa cells stick to oocyte's zona pellucida is apparent.

The extent of pathological changes in the ovarian tissues is more severe in the higher dose (32mg) treated group. The surface epithelium is highly damaged and severely detached and at most of the places absent. The cortical area is filled with numerous primordial follicles extends to the medullary region however, most of them are distorted, exhibits disintegrated nuclei with damaged squamous epithelium and necrosis. There are several interstitial spaces in both cortical and medullary region as the follicles are totally damaged.

Very few primary follicles are found enclosing distorted oocyte with remains of cytoplasm and shrink, indistinct and necrotic granulosa cells. Ovaries of lead treated animals contain both cortical and medullary regions as control but shows damaging pattern in various ovarian components and the distribution of follicles is totally disturbed. During 3rd week in 8mg lead treated group, the surface epithelium is not intact and superficially detached, augmented by total detachment in higher dose level. The granulosa cells of primary and secondary follicles are lightly stained having less dense nuclear material and in preantral follicles, the cytoplasm is dissoluted in antral cavity and distorted theca layers are present at various places. Oocyte is displaced, having nuclear debris in the centre, zona pellucida is also damaged, and far away from cuboidal follicular cells and most of the follicles are without oocyte.

In higher doses (32 mg) the damage is more severe regarding surface epithelium detachment, lesser condensation of nuclei, reduction in the granulosa cells of stratum granulosum with atrophy and smudgy appearance are observed. The oocyte structure is very much affected with irregular zona pellucida, dissolution of cytoplasm and finally disintegration in the ooplasm. In fact the growing phase like preantral and antral is not detected in 32 mg lead treated group at postnatal 14th day.

However, follicle development is not inhibited due to administration of lead but it is examined that as the dose increased the severity of damaging ovarian tissue is more pronounced associated with histoarchitecture and growth of the follicles. The administration of lead severely affects the normal follicular development and produce aberrations in tissue architecture with disrupted surface epithelium, cortical and medullary regions. Although the ovaries exhibits normal folliculogenesis and oocyte development from birth to PND 21, lead dramatically influenced the ovarian histoarchitecture as compared to control.

In lower group (8mg), the surface epithelium surrounding ovarian surface is not continuous and detached at some places, the size of ovary is reduced with decrease in the number of developing follicles in cortical region as compared to the control group ovaries and there is no clear differentiation between both cortical and medullary areas. The primary follicles exhibit dissolution and expansion of cytoplasm, irregularity in zona pellucida and lightly stained nuclear material in the follicles.

The shape of most of the follicles is altered giving oval to squeezing appearance to follicular structure, with slightly hypertrophied and dispersed granulosa cells. There is increase in the number of atretic follicles.

The surface epithelium is highly detached, irregular and plenty of breaks were found at various places. Inter follicular spaces are devoid of stromal cells. In comparison of 1, 7 and 14 days ovary the severity of damage in stromal cells is increased with complete detachment of surface epithelium, severe damage in both cortical and medullary region, wide spaces in stroma, loss of interstitial tissue in matrix, rarely seen blood vessels and detachment of theca cells and extensive alteration in all follicles are detected in 32 mg lead treated group.

Shape of primary, secondary, preantral and antral follicles is altered and looks like bulging or suppressed at one side, oblong and blunt in shape. The stratum granulosum is very severely affected, in primary and secondary follicles all the granulosa cells attached to each other and it appeared like their cytoplasm expels from their cell and showing cytoplasmic dissolution. Oocytes of all the follicles are in degenerated stage, far away from their adjacent granulosa cells, losing their original round shape to distorted structure with dissoluted cytoplasm, at some places it is dispersed throughout the antrum. In preantral and antral follicles granulosa cells are clumped, compact and intermingled with each other, not seen in different layers and their structure is not identified due to the damage. The numbers of atretic follicles are very much increased showing pyknosis in granulosa cells, dissolution in cytoplasm and alteration in structure (**Plate 2 and 3**).

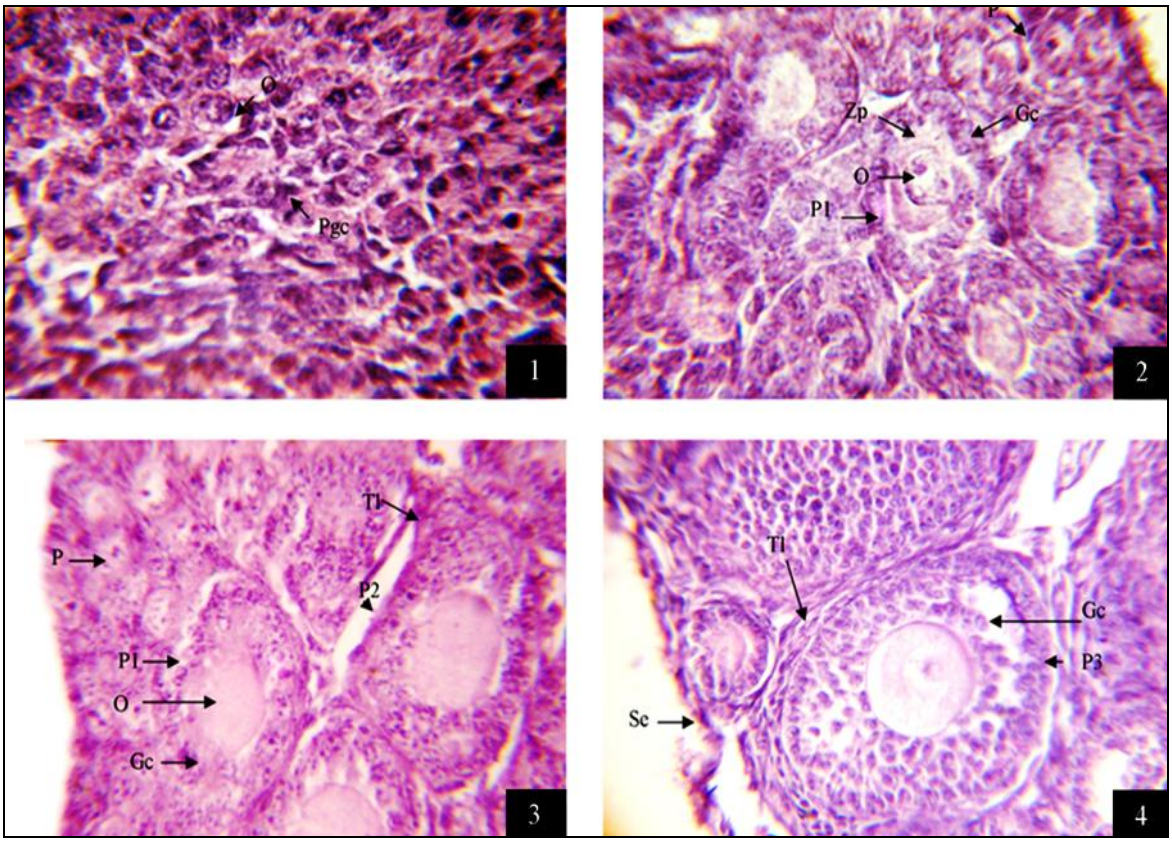

PLATE 2: T.S. OF OVARY OF MICE NEONATE EXPOSED TO LEAD ACETATE (8MG) ON DIFFERENT POST NATAL DAYS (HAEMATOXYLIN & EOSIN STAIN 450X)

Fig. 1: Lead treated (8mg) ovary showing altered structure of germ cells (Pgc) and variation in nuclear material of oocytes (O) on PND 1.

Fig. 2: Compact, compressed ovarian histoarchitecture in lead treated (8mg) ovary on PND 7, showing disarranged and decondensed granulosa cells (Gc) in primary follicle (P1), oocytes (O) with damaged zona pellucida (Zp) showing cytoplasmic dissolution. Primordial follicles (P) are reduced in size and lost their normal distribution.

Fig. 3: Lead treated (8mg) ovary showing damaging pattern in ovarian components on PND 14. Disturbed arrangement of follicles, distorted primordials (P), secondary follicles (P2) having detached zona pellucida (Zp) and irregular distribution of decondensed granulosa cells (Gc) with necrosis in medullary area (M) is visible. Thecal cells (Tl) are also detached.

Fig. 4: Detached and irregular surface epithelium (Se) of lead treated (8mg) ovary showing primary follicles (P1) with damaged and fused granulosa cells (Gc) on PND 21. Tertiary follicle (P3) in cortical area with irregular, scattered and shrinked granulosa cells (Gc) but thecal cells (Tl) are in normal condition.

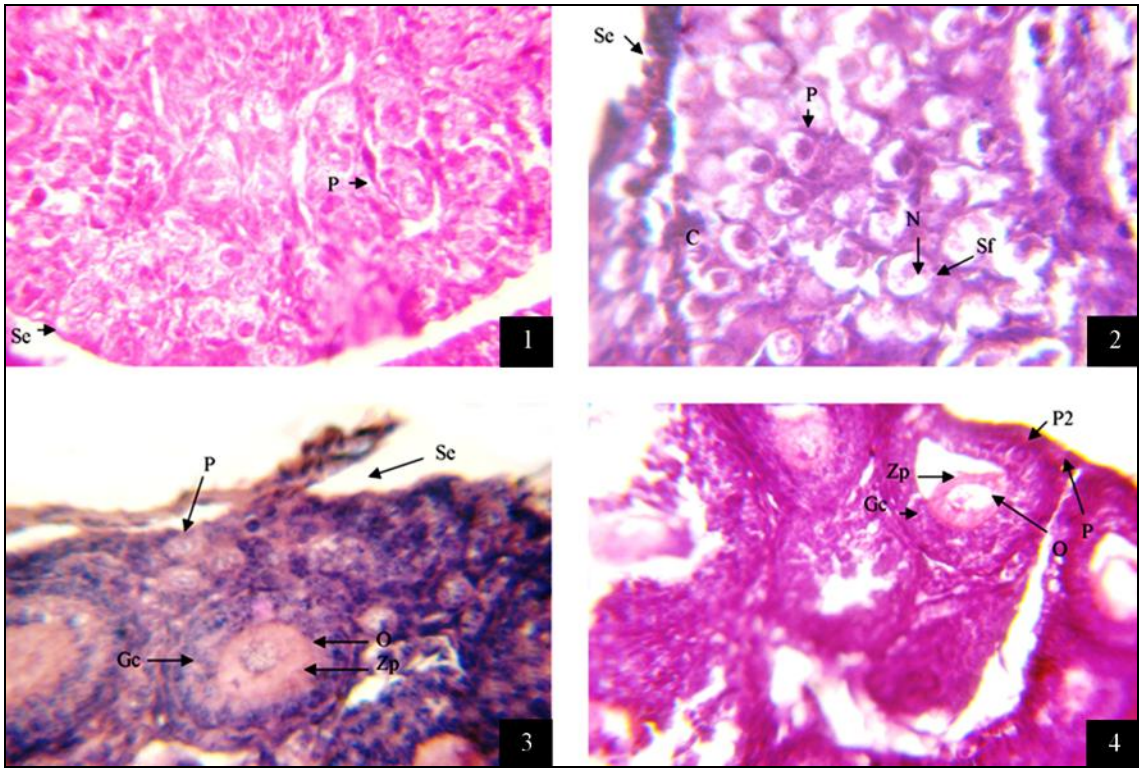

PLATE 3: T.S. OF OVARY OF MICE NEONATE EXPOSED TO LEAD ACETATE (32MG) ON DIFFERENT POST NATAL DAYS (HAEMATOXYLIN & EOSIN STAIN 450X)

Fig. 1: 32 mg lead treated ovary on PND1 showing unclear surface epithelium (Se) and primordial follicles (P) appears in small groups causing gaps in stroma.

Fig. 2: Severely damaged 32mg lead treated ovary on PND 7. Detached surface epithelium (Se), numerous but distorted primordial follicles (P) spreading both in cortical (C) and medullary area (M) showing degenerated nuclei with damaged squamous follicular cells indicates necrosis. Stromal cells are absent.

Fig. 3: Increased damage in 32mg lead treated ovary showing smudgy appearance of ovary on PND 14. Detached surface epithelium (Se), lesser condensation of nuclei and reduction in granulosa cells (Gc) of tertiary follicles (P3)is observed with atrophy. Oocytes (O) are visible with irregular zona pellucida (Zp) and dissolution in cytoplasm.

Fig. 4: Showing compact, suppressed primordial follicle (P) and rectangle shape of secondary follicle (P2) in 32 mg lead treated group on PND 21. The zona pellucida (Zp) is far away from adjacent granulosa cells (Gc) and oocyte (O) with dispersed ooplasm. Suppressed zona pellucida (Zp) and granulosa cells lost their normal architecture showing pyknosis with severe damage in matrix.

DISCUSSION: The ovarian follicle is the fundamental unit of the ovary. It contains the oocyte that may eventually ovulate, undergo fertilization and form an embryo. It also provides the steroid and protein hormones required for maintenance of the ovarian cycle, the secondary sex characteristics and preparation of the uterus for implantation [22].

The development of both the mammalian oocyte and the somatic cell compartments of the ovarian follicle are highly coordinated; this coordination ensures that the ovulated oocyte is ready to undergo fertilization and subsequent embryogenesis. Disruption of this synchrony results in oocyte developmental failure. The histopathological findings of ovary in our study revealed subtle histological changes in the ovaries of newborn mice, showed surface epithelium enclosing differentiating primordial follicle and squamous follicular cells surrounding oocyte and primordial germ cells at birth. The low level lead exposure causes variation in structure of germ cells and nuclear material of oocytes and this damage was higher in high dose 32 mg, where primordial follicles appear in small groups causing gaps in stroma with reduction in germ cells, oocytes and damaged nuclear material.

Similarly Jefferson *et al.*, [23] stated, early in ovarian differentiation, female mouse germ cells develop in clusters called oocyte nests or germline cysts. After birth, mouse germ cell nests break down into individual oocytes that are surrounded by somatic pregranulosa cells to form primordial follicles. Mice treated neonatally with genistein (50 mg/kg per day) on days 1–5, had fewer single oocytes and a higher percentage of oocytes not enclosed in follicles. There was also an increase in the number of oocytes that survived during the nest breakdown period and fewer

oocytes undergoing apoptosis on neonatal day 3. His data suggested that genistein exposure during development alters ovarian differentiation by inhibiting oocyte nest breakdown and attenuating oocyte cell death.

In the present study, the reduced number of germ cells in the ovaries of lead treated embryos may explained by the interference of lead with primordial germ cell migration and/ or delay in the growth of developing gonads. Such interference has been reported in mice embryos which have been injected with mitomycine C [24] and cadmium chloride [25]. Reduction in the number of germ cells in lead treated embryos was also examined by Wide [16]. In consistent to our results Shabaka *et al.,* [26] also studied lead acetate induced reduction of germ cell number in the developing gonads.

Hence, lead acetate is suspected to interfere with the development of gonad and may affect the fertility of the offsprings. This presumption requires further investigation. In our study, in the control group from PND1 to PND21, the number of follicles decreased and very less number of follicles developed in growing phase but lead produced increasing damage in the ovary during these days. The reduction in the number of follicles from 1 to 21 days of postnatal life was increased markedly.

In the present investigation treated females produce babies who have reduced number of different types of follicles and increased number of atretic follicles, the structure of granulosa cells was completely altered day by day, the distinct layers of granulosa cells were reduced with shrinkage in its size, consequently the regular structure of granulosa cells was completely lost. Bires *et al.,* [19] also observed histological changes in the number of ovarian follicles and the increase occurrence of primary atretic follicles indicated alterations in the membrane structures and organelles of oocytes and in the follicular cells of stratum granulosum.

Taupeau *et al.,* [8] reported lead accumulation in low concentration in the ovary caused dysfunction of folliculogenesis with fewer primordial follicles and an increase in atretic antral follicles. Our results are also in conformations with the above findings. Many studies have suggested that not only the high lead dose can damage the ovary [27] but even the low doses has provoked an inhibition in the folliculogenesis leading to the dysfunctions of this process [28] .

The present study showed that neonatal lead treatment diminished the shifting of primordial follicles to primary, secondary and tertiary follicles in the ovaries of mice neonate especially in the higher dose group. Dose-dependent reductions of growing follicles and the presence of higher numbers of primordial follicles (32 mg at PND 7) suggest that neonatal lead treatment inhibits transition from the primordial to primary follicle stage. In agreement of our results Junaid *et al.,* [18] also reported higher number of primordial follicles in neonatal lead treated ovaries as compare to control.

In our study the low lead acetate levels reduced small and medium follicle numbers and high levels resulted in fewer large follicles numbers in mice. Overall, the data suggest that neonatal lead treatment inhibits follicular development in the ovaries of offspring in a dose-related manner. Ercal *et al.,* [29] also observed chronic exposure to lead damaged primordial and medium follicles and arrested follicular development in Rhesus monkeys.

Taupeau *et al.,* [8] illustrated even low doses of lead provoked an inhibition in folliculogenesis leading to dysfunction of this process. It has been reported that lead acetate reduces the number of primordial follicles and is completed by around postnatal day 3 or 4 [30]. During postnatal development some of primordial follicles grew and primary, preantral and antral follicles were seen in the ovaries from 9 to 20 days of age [31].

Chemicals that affect ovarian function can act through direct effects on hormone production or by interference with steroid hormone action (hypothalamus and/or pituitary).

Alternatively, ovarian toxicants can directly cause ovarian failure by extensive follicular destruction. This targeting can result in the loss of ovarian steroid hormones, eventual ovarian failure, and ultimate disruption of neuroendocrine feedback causing increased levels of FSH and LH [32]. Consequently, the present study shows that maternal lead acetate exposure during lactation affects prepubertal ovarian follicle development in a dose dependent manner.

For reproductive toxicants that cause direct damage to ovarian follicles, the stage of development at which the follicle is destroyed determines the impact that exposure to the chemical will have on reproduction [33, 34].

Chemicals that selectively damage large growing or antral follicles only temporarily interrupt reproductive function because these follicles can be replaced by recruitment from the greater pool of primordial follicles. But, our investigation demonstrates lead causing damage to every components and follicle state of neonate's ovary. In low to higher doses of lead surface epithelium detachment is usually observed and that is more prominent in 32 mg lead treated groups. Dumitrescu *et al.,* [35] also reported detachments of epithelial layers in ovary of the female rats.

In the present investigation, along with reduction in the number of medium and large follicles, decrease in number and arrangement of granulosa cells also observed from PND 7 to 14 in different treated groups. In lower doses scattered granulosa cells with shrinkage were clearly visible resulting in hyperplasia and atrophy and the damage was more pronounced in higher doses of lead. 32 mg lead treated group was characterized by fused and indistinct compressed follicles.

Bires *et al.,* [19] also noted histological changes in the number of ovarian follicles and alterations in the follicular cells of the stratum granulosum, reduction in the number of granulosa cells, and apparent shrinkage in these cells were also observed. In most of the follicles the regular structure of granulosa cells altered. The degeneration in granulosa cell was apparent in all developing follicles.

It was postulated that Pb can cause a reduction in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) binding, which significantly alters steroid production in vitro and exerts a direct influence on granulosa cell function [21].

Though heavy metals lowers the growth potential of oocytes at a concentration higher than that present in the environment, their influence on female infertility could not be ruled out due to its cumulative effect or their chronic exposure [36]. Atretic follicles from primary through antral follicles display pyknotic, darkly staining granulosa cells losing the contact to the oocyte. At the same time the proliferation activity of granulosa cells decreases during follicular atresia [37] a process that could be confirmed [38]. These findings were also in confirmation of our results. The primary mechanism of the toxic action of lead appears to be a disruption of the hypothalamic control of pituitary hormone secretion [39].

Oxidative damage associated with the presence of lead has been illustrated as one possible mechanism involved in lead toxicity [40], Animals have protective mechanism in the form of antioxidant nutrients, vitamins and several enzymes. Antioxidant may play an important role in abating some hazardous effects of lead.

Alternatively, ovarian toxicants can directly cause ovarian failure by extensive follicular destruction. This targeting can result in loss of ovarian steroid hormones, eventual ovarian failure (menopause), and ultimate disruption of neuroendocrine feedback causing increased levels of FSH and LH [5]. In the mouse, follicular formation and development is mostly postnatal. Changes in ovarian steroidogenesis during early postnatal life can alter folliculogenesis in adults.

CONCLUSION: Lead is a strong teratogen which causes most of its congenital effect at the time of organogenesis during embryonic period. The results of present investigation clearly emphasize that prenatal lead exposure is extremely dangerous and a strong correlation between maternal and umbilical cord blood lead levels indicating prenatal transfer of lead from mother to developing fetus in uterus. Lead suppresses the development of primordial follicles during fetal and neonatal life. Severe pathological changes in primary and secondary follicles with increased number of atretic follicles were also observed.

Inorganic lead is a suspected developmental toxicant and possibly has adverse effects on the developing ovary through action on the hypothalamic pituitary axis or by disrupting the delicate pro-oxidant or antioxidant balance that exists within mammalian cells during neonatal life. We concluded that lead acetate has dose dependent toxic effects on development of ovarian follicles of neonatal mice. It is suggested that exposure to lead in female during gestation and lactation period exerts a negative impact on fertility by interfering with the normal development of the ovary of mice neonates during early period of organogenesis.

ACKNOWLEDGEMENT: Authors are thankful to the Department of zoology, college of science, M. L. S. University for providing necessary facilities for the present piece of research work.

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How to cite this article:
Sharma R, Panwar K, Barber I and Purohit A: Lead toxicity and postnatal development of Ovary. *Int J Pharm Sci Res* 2013; 4(4); 1575-1584.