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Research Article

Effect of Exposure to Mixture of Heavy Metals Arsenic, Cadmium and Lead on Reproductive Function and Oxidative Stress in Female Rats

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ABSTRACT

Background : Toxicological consequences arising from exposure to mixtures of heavy metals especially at low, chronic and environmentally relevant doses are poorly recognised. In the present study, we evaluated effects of chronic exposure to combinations of three metals arsenic (As), cadmium (Cd) and lead (Pb) present frequently in drinking water on reproductive function and oxidative damage caused to reproductive organs of female rats. Method : Female rats were exposed to mixture of metals (As, Cd& Pb) for 90 consecutive days. The gain in body weight and weight of reproductive organs were recorded following autopsy on 91 stday. The oestrus cycle were monitored during entire treatment period. Numbers of corpora lutea, implantation sites, live fetus and survival of the fetus were evaluated in rats mated successfully with untreated male after completion of their respective treatment. Ovarian cholesterol, protein, ascorbic acid and enzyme Δ^5-3β HSD levels were estimated. Serum levels of steroid hormones oestrogen and progesterone were estimated. Histopathological picture of both ovary and uterus were assessed. Levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPX) activity, amount of reduced glutathione (GSH) and malondyaldehyde (MDA) in blood, ovary and uterus were measured as biomarkers of oxidative stress. Results : The treated rats showed reduced body weight gain and reduction in the weight of ovary and uterus. Oestrus cycle was disrupted with continuous diestrous in treated animals. Number of corpora lutea, implantation sites and live fetus and the survival of fetus evaluate were reduced significantly in treated groups. The levels of ovarian cholesterol and ascorbic acid increased in treated rats with decrease Δ^5-3β HSD level. There was reduction in serum level of both the ovarian steroid hormones oestrogen and progesterone. The protein levels did not differ between the groups. There was a significant increase in levels of MDA and decrease in levels of all the antioxidant enzymes in treated group. Conclusion : The results revealed there was disruption to reproductive functions with decrease in stereoidogenic activity and associated oxidative stress in female rats treated with combination of mixture of metals (Cd, As and Pb) at low dose for 90 consecutive days.

Keywords: oestrus cycle, implantation, steroidogenesis, oxidative stress.

INTRODUCTION

The toxicity caused by heavy metals has become a serious health concern worldwide that adversely effects along with other organ systems of the body the reproductive health of various living organisms[1]. Most of the toxicological evolutions of metals restrict to a single metal and at a very high exposure dose. However, both animals and human are exposed concurrently and chronically to mixture of heavy metals through variety of sources including drinking water. Information on chronic low dose exposure to a mixture of heavy metals is rarely available. We, therefore, designed the experiment to evaluate chronic low dose exposure to a mixture of potentially toxic and environmentally relevant heavy metals-arsenic (As), cadmium (Cd) and lead (Pb) in female reproductive system.

Arsenic (As) is a potent water contaminant and is reported to be present in high concentrations in water of South East Asian countries including India[2]. Developmental effects, cancer and cardiovascular disease have all been associated with long-term exposure to arsenic (As)[3]. Arsenic (As) caused male reproductive toxicity when given through drinking water[4]. It can induce ovarian and uterine toxicity and can influence neuroendocrine regulation of female sex hormones. Arsenic (As) can be transferred from the mother to the fetus and thus can cause developmental toxicity. However, most of these studies lack detailed information on cofounders and their probable mode of action.

Exposure to low levels of cadmium (Cd) through food and water is common in different areas. It elicits diverse toxic effects including nephrotoxicity, carcinogenicity, teratogenecity and immunotoxicity. Cadmium (Cd) has also been shown to cause reproductive toxicity either directly targeting gonads or indirectly by interfering with hypothalamopituitary gonadal axis. Acute cadmium (Cd)

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exposure in pregnant rats induced placental necrosis and increased fetal death[5]. Reports on long term chronic exposure to cadmium in female rats in combination with other environmental pollutants are scares.

Environmental and occupational exposure lead (Pb) is still a major public health problem for most of the developing countries[6]. Lead (Pb) enters human body mainly through ingestion and inhalation. Chronic exposure to lead (Pb) is associated with various neurological, haematological, immunological and hepatic disorders. It is reported to effect male reproductive system adversely causing altered spermatogenesis and testicular degeneration[7]. Severe cases of lead (Pb) poisoning have been reported to be associated with sterility, miscarriage, abortion, premature delivery and infant mortality[8]. Lead (Pb) is reported to cross the placenta during pregnancy and is associated with various pregnancy related defects. There is limited laboratory studies on the impact of chronic low dose co-exposure to lead (Pb) in female.

Many studies suggested that generation of reactive oxygen species (ROS) and it's interference with cellular antioxidant system is one of the major mechanism by which toxic effects of metals are mediated[9]. However, there are very few studies that examined the oxidative stress in reproductive organs exposed to a mixture of heavy metals arsenic, cadmium and lead. In view of the above facts, the aim of the present study was to address the effect of long term exposure to combination of As, Pb and Cd at low dose on reproductive function and oxidative stress parameters in female albino rats.

METHODOLOGY

Animals

Female albino rats of Wister strain (160-170g, body weight) were used for this study. The rats were housed in the animal house facility of Tripura University in clean and disinfected plastic cages and were fed with a standard rat food and allowed to drink water *ad libitum*. They were maintained in a controlled condition of temperature (25± 2˚C) and normal day/night schedule (12L: 12D). Ethical clearance for the study was obtained from Animal Ethical Committee of Tripura University.

Experimental design

The oestrus cycle of the rats were checked daily at 10 A.M. to 11 A.M. for 15 days. After 15 days forty eight (48) female albino rats with regular oestrus cycle were selected for the experiment. The animals were divided into two groups – control and treated with equal number of rats[24] in each group. The control animal received 2ml water orally through gavages for 90 consecutive days. The treated group received mixture of arsenic (As) cadmium (Cd) and lead (Pb) and were orally for ninety (90) days. The doses of drugs were determined on the basis of our previous findings on the combined effect of mixture of metals As, Cd and Pb and on reported base line dose of metals in drinking water[10] . The oestrus cycle of all the animals were checked through the entire period of the experiment.

After ninety (90) days of respective treatment, twelve (12) rats from each group were sacrificed on 91st day by cervical dislocation following ether anaesthesia. Utmost care was taken during the time of sacrifice according to Indian Council of Medical Research (ICMR) guidelines[11].

Other twelve (12) rats from each group were allowed to mate with untreated males on their respective oestrous day in the 1:1 ratio. The rats were then cheeked for presence of vaginal plugs or spermatozoa in the vaginal orifice, as evidence of mating. After successful mating, the male were removed from the cage. The day when vaginal plugs or spermatozoa were observed was considered as gestation day (GD0). To assess the implantation loss, half of the animals in each group were sacrificed on GD18 to observe the number of corpora lutea of pregnancy, number of implantation sites and status of the embryo. The remaining rats were allowed to complete their gestation and pups born were observed for another 90 days.

Recording of Body weight and organs weights:

The weight of each rat was taken on the first day of the experiment and at the interval of seven days during the entire period of experiment. The body weight taken on the 1st day of experiment was considered as the initial body weight. The body weight taken before autopsy was considered as final body weight. After autopsy the ovary and uterus were dissected out and freed from adherent tissues and blood vessels, blotted free of mucous and weighed to the nearest milligram. The organ weights were expressed per 100g body weight to ensure normalization of data for statistical analysis.

Collection of blood and organs

Blood was collected by puncturing the heart. Heparin (2 mg/ml) was used as an anticoagulant. Blood samples were centrifuged at 2000 rpm for 15 min to separate plasma and stored at -20^{O}C for further analysis. Ovary and uterus and other vital organs from the control and experimental animals were quickly excised. The organs were washed in ice-cold saline and blotted by Whatman No.1 filter papers for drying. Ovary and uterus were kept at -80ºc for further biochemical analysis and preparation of permanent slides for the histopathological study.

Preparation of tissue homogenate and separation of erythrocytes

Ovary and uterus were homogenized in 20% glycerol, 5mμ potassium phosphate, 1mμ EDTA in 4:1 volume buffer to organ weight at 4^{0}C by using a Teflon homogenizer.The layer of white blood cells above the packed erythrocytes was discarded. Erythrocyte pellet was washed three times with 0.15 M NaCl, diluted (33%) in phosphate buffer saline (mM: NaCl, 136.9; KCl, 2.68; KH2PO4, 1.47; and Na2HPO4, 6.62; pH 7.4) and kept at 4^{0}C until further analysis. The 33% packed erythrocytes were used for the estimation of LPO, GSH. Hemolysate (10%) was prepared by diluting the packed erythrocytes in phosphate-buffered saline (PBS) and used for the determination of activities of different antioxidant enzymes. Following parameters were analysed in both control and treated animals:

Determination of Protein content

The protein content in ovary and uterus was determined according to the method of Bradford et al., 1976[12].

Determination of Cholesterol content

Table 1: Effect of mixture of heavy metals (As,Cd&Pb) on body weight gain and weight of reproductive organs of the animals.

Parameters	Group-I : Control (12)	Group-II : Treated (12)
Body weight gain (%)	98.98±0.429	63.60±0.526**
Weight of ovary (g/100gbw)	0.28±0.008	0.12±0.008**
Weight of uterus (g/100gbw)	4.15±0.033	2.14±0.010**

Number in parenthesis indicates no. of animal in each group. , Values represent mean ± SE of twelve (12) rats; *= p<0.05; ** = P< 0.01; *** = p<0.001 ; #= non-significant

Table 2: Effect of treatment with Mixture of Metals on implantation of the animal and survival of foetus.

Parameters	Group-I : Control (06)	Group-II : Treated (06)
No. of corpora lute	12.666±0.210	10.500±0.341**
No. of implantation	11.333±0.210	8.333±0.210**
Pre-implantation loss (%)	21	71**
No. of live foetus	9.5±0.561	6.166±0.166**
Post implantation loss (%)	17	81**
No. of pups	8.92±0.68	6.24±0.84**
Survival at birth time	100%	100%$^{\#}$
Survival after 30 days	90%	31%**
Survival after 60 days	90%	31%**
Survival after 90 days	90%	25%**

Number in parenthesis indicates no. of animal in each group. Values represent mean ± SE of six (06) rats; p <0.05; ** = P< 0.01; *** = p<0.001, #= non-significant

The ovarian cholesterol content was determined according to the method Zlatkis et al., 1953[13].

Determination of ascorbic acid content

The ascorbic acid content of ovary was estimated by method of Roe and Kuether, 1943[14].

Estimation of Δ^5 3β-HSD activity

The Δ^5 3β-HSD was determined in tissue (ovary) according to the method of Talay et al., 1962[15].

Estimation of serum level of ovarian steroid hormone oestrogen and progesterone

All hormones were quantified by liquid-phase radio immunoassay (RIA) using double antibody technique following the procedure mentioned by Sufi et.al., 1985[16].

Determination of Malondyaldehyde (MDA) concentration

Table 3: Effect of treatment with Mixture of Metals on concentration (µg/mg tissue) of different biochemical parameters in ovary of the animals.

Parameters	Group-I Control (12)	Group-II Treated (12)
Cholesterol (mg/gm of tissue)	12.17±0.17	14.50±0.22***
Ascorbic acid (mg/100gm of tissue)	184.33±0.33	214.50±0.50***
Protein (µg/mg of tissue)	151.33±0.210	150.41±0.436$^{\#}$
Δ^5-3β-HSD activity (unit/mg of tissue/hr)	32.50±0.22	22.33±0.33***

Number in parenthesis indicates no. of animal in each group ; Values represent mean ± SE of twelve (12) rats; *= p <0.05; ** = P< 0.01; *** = p<0.001, #= non-significant.

The malondialdehyde concentration was determined in ovary, uterus and blood according to the method of Ohkawa et al., 1979[17].

Determination of catalase activity

The catalase activity was determined in ovary, uterus and blood according to the method ofAebi, 1984[18].

Determination of superoxide dismutase (SOD) activity

The superoxide dismutase activity was determined in ovary, uterus and blood according to the method of Marklund and Marklund et al., 1974[19].

Determination of reduced glutathione (GSH)

The reduced glutathione was determined in ovary, uterus and blood according to the method of Ellaman et al., 1959[20].

Determination of glutathione reductase activity

The glutathione reductase activity was determined in ovary, uterus and blood according to the method of David and Richard, 1983[21].

Determination of glutathione peroxidase (GPx) activity

The glutathione peroxidise activity was determined in ovary, uterus and blood according to the method of Paglia and Valentine, 1967[22].

Histopathological study

The ovary and uterus were fixed in 4% formalin solution for 72 hrs. The fixed organs were dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax, and then 4-5 µm thick sections were obtained by rotary microtome and stained by Harris haematoxylin and Eosin. The stained section of the organs was examined under low (20X) and high power (40X) of binocular microscope. The clean and clear photomicrographs of control and experimental ovary and uterus were taken.

Data analysis

All parameters were statistically evaluated using Student's t test to determine the differences between control and treated groups (p<0.05).

RESULT

Body weight gain and weight of reproductive organs

Table 4: Effect of treatment with Mixture of Metals of antioxidant enzymes-Catalase, SOD, GSH, GR and lipid peroxidation levels in erythrocytes, ovary and uterus of female rats.

Parameters	Organs					
	Erythrocytes		Ovary		Uterus	
	Group-I : Control (12)	Group-II : Treated (12)	Group-I : Control (12)	Group-II : Treated (12)	Group-I : Control (12)	Group-II : Treated (12)
Catalase (µmol H_2O_2 Consumed/min/mg protein	136±0.673	102.05±1.453**	4.58±0.083	3.58±0.016**	1.42±0.107	0.31±0.018**
Superoxide Dismutase (µmol/mg protein)	6.07±0.04	4.32±0.11**	2.54±0.009	1.44±0.018**	1.03±0.021	0.71±0.031**
Reduced Glutathione (µmol/mg protein)	0.44±0.007	0.29±0.009**	2.92±0.009	1.80±0.025**	1.65±0.038	0.061±0.810**
Glutathione Reductase (nmol/NADPH consumed/min/mg protein)	29.79±0.637	21.25±0.367**	2.183±0.194	0.562±0.006**	1.44±0.106	0.60±0.037**
Glutathione Peroxidase (Unit/mg protein)	21.52±0.27	16.50±0.230**	21.52±0.27	16.50±0.23**	0.92±0.011	0.76±0.009**
MDA Concentration (nmol/mg protein)	4.62±0.044	7.52±0.109**	2.74±0.061	11.88±0.439**	27.68±0.203	51.14±2.026**

Number in parenthesis indicates no. of animal in each group ; Values represent mean ± SE of twelve (12) rats. $p < 0.05$; ** = $P < 0.01$; *** = $p<0.001$, #= non-significant.

There was significant reduction in body weight gain in treated group compared to control group. The weight of ovary and uterus was also decreased significantly in treated rats in comparison to the control animals (Table-1).

Estrous cycle

Studies on oestrous cycle revealed female rats treated with mixture of heavy metals arsenic, cadmium and lead had prolonged diestrous with significant reduction in proestrus and oestrus phases of the cycle (Fig 1).

Implantation and development of foetus

Treatment of female rats with the mixture of metals resulted into significant pre and post implantation loss in comparison to the control animals (Table 2).

There was a significant decrease in number of pups born to the treated female and 69% of the pups died within 15 days after parturition (Table 2).

Steroidogenic parameters

There was a significant increase ($p<0.01$) in concentration of cholesterol and ascorbic acid level in ovary of mixture of metals As, Cd & Pb treated animals in comparison to animals of control group .The concentration of protein in ovary did not vary significantly between animals of treated and control groups . There was a significant decrease ($p<0.01$) in concentration of Δ^5-3β-HSD in ovary of mixture of metals treated animals in comparison to animals of control group (Table 3). There was significant decrease in serum levels of both the steroid hormones oestrogen and progesterone in treated rats (Fig. 2 A & B).

Oxidative stress parameters

There was a significant decrease ($p<0.01$) in the activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) activity in ovary, uterus and erythrocytes. Reduced glutathione level also reduced significantly in treated animals. However, there was an increase in ($p<0.01$) in lipid peroxidation marked by increased level of malondyaldehyde in blood and ovary of animals treated with mixture of metals, arsenic (As), cadmium (Cd) and lead (Pb) compared to animals of control group (Table 4).

Histopathological study

Ovarian picture of treated animals showed signs of ovarian damage such as distortion of attritic follicles, distortion of follicles, distortion of medulla, distortion of theca and zonagranulosa of the ovary (Fig 2 C & D) in comparison to control animal (Fig 2 A & B).

Uterus of the treated animals showed signs of damage like endometritis, narrowing of vaginal lumen and distortion of endometrial gland(Fig 3 C & D) in comparison to control animal (Fig 3 A & B).

DISCUSSION

The arsenic (As), cadmium (Cd) and lead (Pb) are hazardous xenobiotics, commonly found in our environment. They induce several physiological, biochemical and histological alterations in living organism including human being[23]. These three metals seem to act

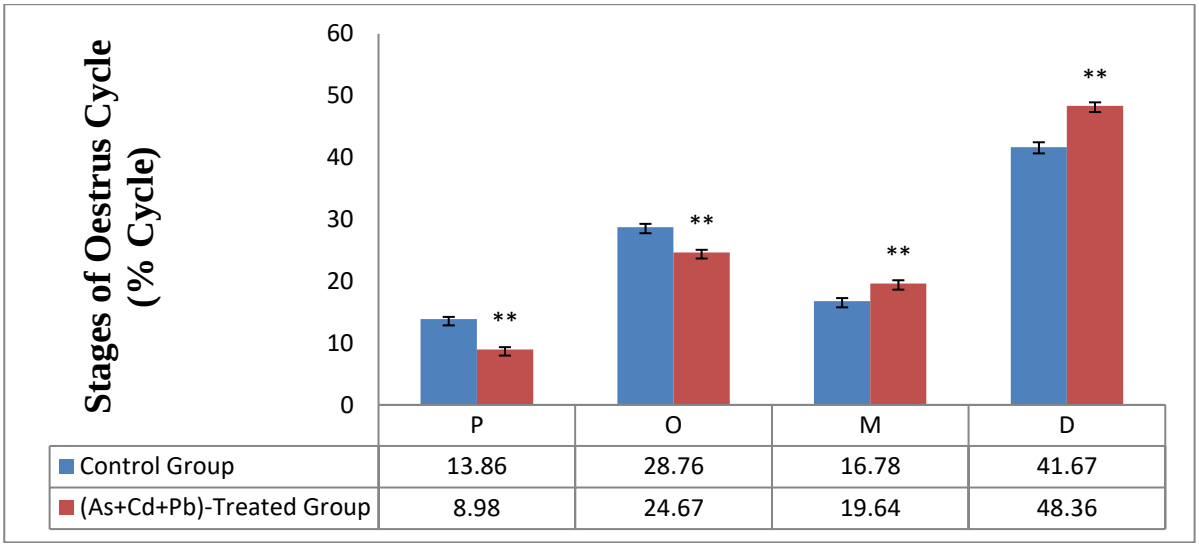
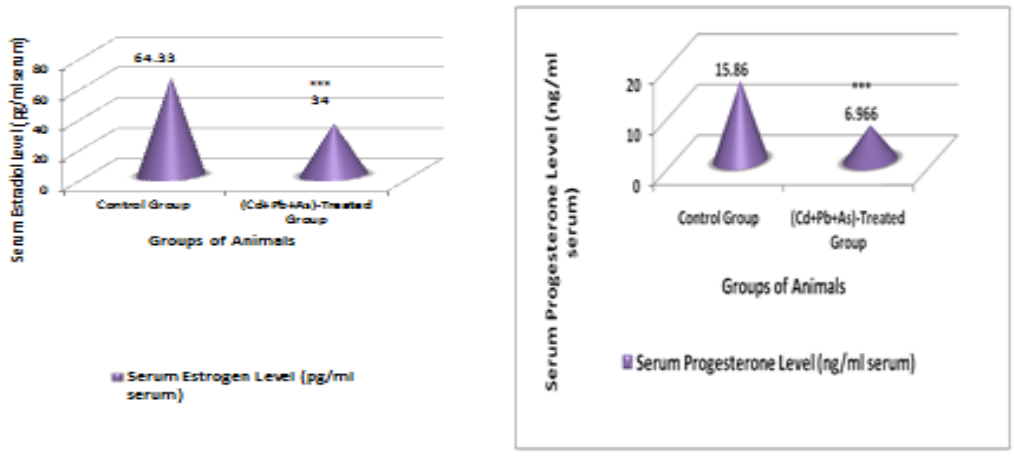


Figure 1: Effect of treatment with Mixture of Metals on oestrus cycle of the animal.
Values represent mean ± SE of twenty four (24) rats in each group;
* = p <0.05; ** = P< 0.01; *** = p<0.001; #= non-significant



2 A

2 B

Figure 2: Effect of treatment with mixture of metals (Cd, Pb & As) on serum levels of steroid hormones : Fig 2A – Oestrogen level ; Fig 2B – Progesterone level.
(ng/ml) level of the animals.
*- p<0.05 ;**- P<0.01 ;*** P < 0.001 ; #- Non significant.

synergistically to induce developmental neurotoxicity spanning in utero to postnatal development in rats[24].

In our study, a significant decrease in body weight gain, weight of ovary and uterus were observed in animals treated with mixture of metals, arsenic (As), cadmium (Cd) and lead (Pb) at a low environmentally relevant dose for 90 consecutive days. Decreased body weight gain in exposed animals relative to control indicated the growth retarding effect of the mixture of these three heavy metals on female albino rats. Reports suggested reduction in food and water intake might contribute to decrease in body weight gain in exposed animal[25]. The reduction of weight of reproductive organs ovary and uterus was indicative of toxic effect of the metal mixture on reproductive system of female rats treated with metal mixture.

This was further supported by our observation on the effect on oestrus cycle of both treated and control groups. Studies on oestrous cycle revealed that sub chronic treatment with mixture of heavy metals As, Cd and Pb caused prolongation of diestrous with decrease in proestrous and oestrous phase in the reproductive cycle of treated female rats. Similar prolongation of diestrous and proestrous are observed in rats by various investigators after treatment with heavy metals[26]. The findings on implantation showed a significant pre implantation and post implantation loss in treated animals with decrease in the number of live foetus. Survival study of animals born to the treated female showed only 30% of animals survived after 15 days of birth. Dhir observed similar effects on progenies of pregnant rats exposed to cancer causing heavy metals Cu, Cd and Pb[27].

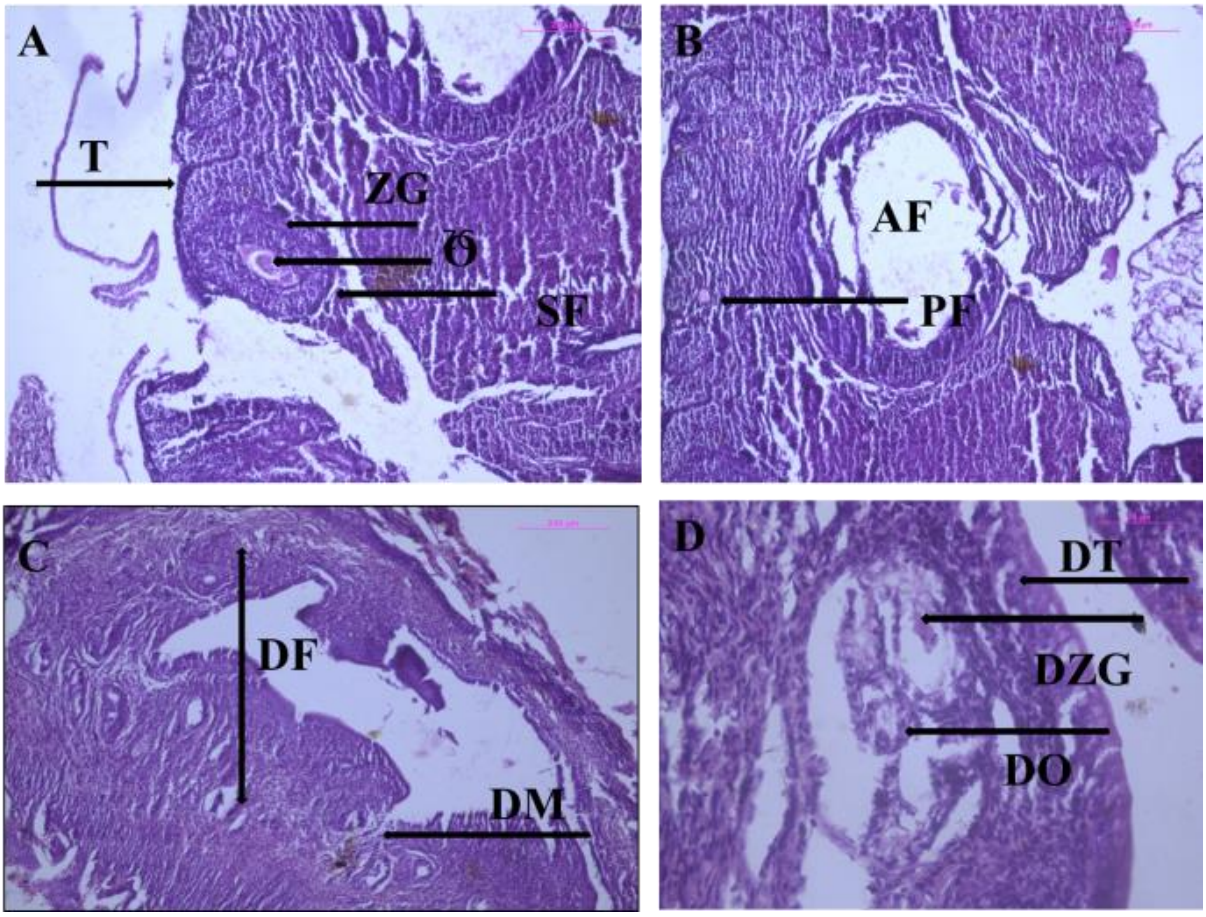


Figure: 3A and B (20x and 40 x) ovary of control group: showing normal structure.
AF- Ataractic follicles; O- Ooctye; PF- Primary follicle; SF- Secondary follicle
ZE- Zonagranulosa
3C and D (20X and 40X) ovary of treated (As+ Pb+ Cd) group: showing distorted structure of ovary.
DAF- Distortion of ataractic follicles; DF- Distortion of follicles; DM- Distortion of medulla: DO-Distortion of ovum ;DT and DZG- Distortion of theca and zonagranulosa.

As we know, cyclic changes in cellular characteristics during different phases of oestrous cycle depends entirely on steroid hormones, disruption in normal cyclic changes of vaginal smear can, therefore, be taken as an indication of steroidal deficiency. Further, reduction in the weight of steroid dependant organ ovary and uterus also suggested that the chronic treatment with the metal mixture caused decrease in normal steroid levels in treated animals which ultimately resulted in reduced growth of steroid dependant organs ovary and uterus. This was also supported by the histological pictures of both the organs. The observations were corroborated by our findings of decline in the serum levels of both the ovarian steroid hormone in treated animals.

In connection to biochemical mechanism of steroid hormones, we measured cholesterol, the chief steroid precursor, and observed a significant accumulation of cholesterol in steroid synthesizing organ, i.e., ovary of the treated rats. Ascorbic acid, though, is not directly working as a precursor of steroid but is essential for the synthesis of steroid[28]. Our observation also showed accumulation of ascorbic acid in ovary of treated animals. This suggested less utilization of both cholesterol and ascorbic acid for biosynthesis of steroid hormones by the animals treated with the metal mixture.

Δ^5-3β-HSD is the key enzyme in the synthetic pathway of steroid hormones in female. Changes in their levels in a tissue indicate strongly the alteration in steroidogenesis[29]. A positive correlation had been established between Δ^5-3β-HSD activity and steroid hormone production. In our observation, we found significant decrease in the levels of this enzyme in mixture treated animals, which was a clear indication of decreased steroidogenesis in animals exposed to the metal mixture. This interference of steroid production was thought to be the main cause of reproductive toxicity of the metal mixture. Similar decrease in Δ^5-3β-HSD activity leading to changes in reproductive function was observed by Sangha et. al. in cypermethrin treated female rats[30].

Successful implantation and development of an embryo in utero involves complex series of synchronized biochemical and biophysical changes in both the blastocysts and the endometrium. To ensure implantation, hormone dependent changes in uterus leading to development of a "receptive endometrium" is a necessity. An essential component of endometrial receptivity is the

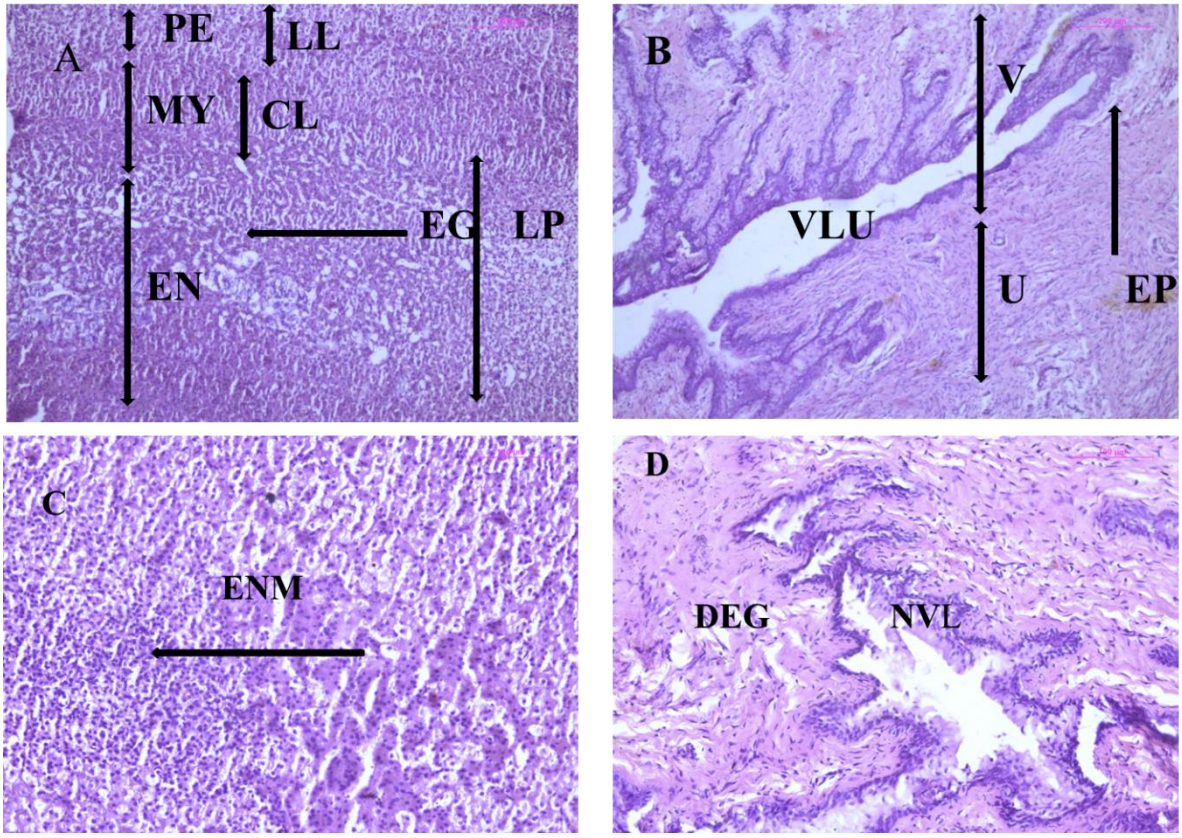


Figure: 4 A and B (20x and 40 x) uterus of control group: showing normal structure of uterus.
EN- Endometrium; M- Myometrium ; PE- Perimetrium; EG- Endometrial gland;
CL- Circular layer ;LL-Longitudinal layer V- Vagina; U- urethra; EP- Epithelium;
VLU- Vaginal lumen ;LP- lamina propia
4 C and D (20X and 40X) uterus of treated (As+ Pb+ Cd) group: showing distorted structure of uterus.
ENM-Endometritis; NVL- Narrowing of vaginal lumen; DEG- Distortion of endometrial gland.

development of its potential for a decidual cell transformation reaction. In our experiment, reduction of implantation sites on GD18 along with significantly reduced weight of uterus in mixture treated animals supported the view that the metal mixture along with its interference with steroid hormone synthesis had an inhibitory influence over initiation and/or maintenance of decidual cell reaction in uterus[31]. There were significant decrease in number of functional corpora lutea in mixture treated pregnant rats, this suggested that the treatment might have interfered with production of progesterone – the hormone of pregnancy, without which the gestation can hardly start, and pregnancy can not be maintained without its continued production[32]. This inhibitory influence of the metal mixture was also evident by the histological picture of the uterus which registered poorly developed endometrium in treated animals.

It was reported that many of the adverse effects of heavy metals in different organ systems were due to change in oxidant/antioxidant balance producing oxidative stress[33]. The results of our study demonstrated, in blood and in both the reproductive organs ovary and uterus of animals treated with the metal mixture, there was an increase in the levels of MDA which is a prooxidant, while the levels of antioxidants such as catalase, SOD, GPx and GSH were decreased.

The female gonads are highly susceptible to the damages induced by reactive oxygen species due to presence of polyunsaturated fatty acids in their cell membranes[34]. MDA, the end product of lipid peroxidation is known to be the most harmful product of free radicals in the cell[35]. Reports suggested that ovarian tissue is particularly susceptible the damage caused by increased MDA produced by various chemical stressors[36]. It was also reported that in ovarian oxidative damage occurring due to ischemic reperfusion, MDA level was significantly increased in comparison to the healthy ovarian tissue[37]. Therefore, increased MDA levels observed in blood, ovary and uterus of treated rats in our study indicated to the oxidative damage caused to these tissues due to treatment with the metal mixture.

However, the biological systems are protected from the oxidative damage of reactive oxygen species by the anti-oxidative defence system, including enzymatic and non-enzymatic scavengers. The free radical scavenging enzymes are catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-s-transferase (GST). They are first line of cellular defence against the toxic effects of ROS[38]. Catalase (CAT) and superoxide dismutase (SOD) mutually function as important enzymes in the elimination of ROS. Superoxide

dismutase (SOD) catalyzes the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen, while catalase (CAT) catalyzes the breakdown of toxic hydrogen peroxide (H_2O_2) to water (H_2O_2) and oxygen (O_2)[39]. Glutathione (GSH) acts as a natural antioxidant and potential reducing agent. It protects the body systems from damaging effects of oxidative stress .In healthy cells and tissue, more than 90% of the total glutathione exists in reduced form (GSH) and less than 10% is in the disulfide form (GSSG). GSH is capable of reducing any disulfide bond formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent (H + e) to other unstable molecules, such as ROS. In this process, GSH is converted to its oxidized form, glutathionedisulfide (GSSG) by the enzyme glutathione peroxidase, GPx and the oxidized form of glutathione reverts to reduced form of glutathione (GSH) by the enzyme glutathione reductase (GR). GSH is found almost exclusively in its reduced form, since the enzyme that reverts it from its oxidized form, glutathione reductase (GR), is constitutively active and inducible upon oxidative stress. Therefore, the decreased ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) within cells is often used scientifically as a measure of cellular toxicity or an indicative of OS generation in the cells or tissues[40].

In the present study, the female reproductive organs, ovaries and uterus in metal mixture treated rats for 90 days showed significant decrease in the catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione reductase (GR) enzyme activities. These observations indicated that combined exposure to three metals inhibited antioxidant enzyme activity and reduce the overall efficiency of the antioxidant enzyme system which lead to cellular dysfunction of ovarian and uterine tissues in treated animals. Recently Markiewicz-Górka et al, in their study reported increased production of reactive oxygen species resulting in cellular damage in hepatic and cardiac tissues of mixture of metals lead (Pb) cadmium (Cd) and manganese (Mg) treated rats[41]. Increased production of reactive oxygen species along with reduced activity of antioxidant enzyme system in metal mixture treated rats in our study indicated that the reproductive dysfunction produced by exposure to the metal mixture might be due to the oxidative damage.

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