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RESEARCH ARTICLE

CYTOMODULATORY EFFECT OF *LACTOBACILLUS SPOROGENES* ON OVARY OF ARSENIC EXPOSED MICE.

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Abstract

Arsenic compounds have been widely used in pesticides, herbicides and soil disinfectors, thus in some soils, its concentration was very high. Groundwater arsenic concentration was increasing in many parts of the world in last few decades. Arsenic is a highly toxic metal element that annually threatens the health of millions of people in the world. The increase of these pollutants in the environment is considered as a serious threat to human and environmental health. Groundwater arsenic contamination was high in many countries including India. The microbial processes for bioremediation of toxic metals employ living cells, non-living biomass or biosorbents. A wide variety of fungi and bacteria are now under study. Thus the present study is designed to evaluate the cytomodulatory effect of *Lactobacillus sporogenes* on ovary of arsenic-exposed mice. Sodium arsenate was administered 5 mg/kg b.wt for 4 weeks was followed by the administration of *Lactobacillus sporogenes* for 8 weeks at 15 million spores/kg body weight. Serum was collected for hormonal study. The ovary was fixed for light microscopic study. Estrogen level was increased 30 folds in the arsenic administered group of mice. Degenerated germinal epithelium and corpus luteum were observed. Degenerated ova and mature Graffian follicle were also observed in the arsenic administered group. Lactobacillus causes marked restoration in estrogen level and ovarian follicles including ova. It is concluded from the entire study that Lactobacillus causes effective restoration in estrogen level. It also maintains Germinal epithelium, follicular stages, corpus luteum and ova in the ovary of mice. This indicates that *Lactobacillus* maintains normal female fertility in mice.

Introduction:-

Heavy metal pollution is increasing nowadays and it is one of the most important environmental concerns. Anthropogenic activities like metalliferous mining and smelting, agriculture, waste disposal or industry discharge a variety of metals such as, which can produce harmful effects on human health when they are taken up in amounts that cannot be processed by the organism. Damage may cause adverse reactions in different organs and biological functions, including reproductive and birth defects (Malik, 2004). Arsenic has long been an important environmental

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pollutant and in long term, it has been as a health risk to humans and other living organisms. Arsenic compounds have been widely used in pesticides, herbicides and soil disinfectors, thus it was the high concentration in some soils (Pais and Banton, 1997). Arsenic is a highly toxic metal element that annually threatens the health of millions of people in the world (Chen and Shao, 2009). Increase in these pollutants in the environment is considered as a serious threat to human and environmental health (Banaa et al, 2010).

In recent decades following an increase in environmental pollution by heavy metals, scientists attracted to biological purification methods. In most cases of cleaning the contaminated ecosystems with chemical methods involves heavy costs and irreparable damages (Brookes, 1995; Nwuche and Ugoji, 2008). Arsenic-resistant bacteria play an important role in controlling the speciation and cycling of arsenic in the ecosystems (Inskeep et al, 2007). Pepi et al, 2007, isolated three arsenic resistant genera (*Aeromonas*, *Bacillus* and *Pseudomonas*) from contaminated sediments with the MIC of 16.66 mM (arsenite) and 133.47 mM (arsenate). They also concluded that these bacteria are suitable for arsenic bioremediation in contaminated sediments. In a study by Luis et al, 2006, in Spain with the aim of biological removing of arsenic, *Corynebacterium glutamicum* with over 60 mM arsenite resistance.

A characteristic component of Gram-positive bacteria cells is teichoic acids and acids associated with the cell wall, whose phosphate groups are key components for the uptake of metals. Few works consider these interactions at the molecular level (Beveridge, 1989; Da Costa, 1999). Carboxyl groups are the main agents in the uptake of heavy metals. The sources of these carboxyl groups are the teichoic acids, associated to the peptidoglycan layers of the cell wall. Microbial biomass offers an economical option for removing heavy metals by the phenomenon of biosorption (Gupta and Mohapatra, 2003). Thus this property of bacillus can be used for the accumulation of heavy metals polluting the environment.

The microbial processes for bioremediation of toxic metals were practiced widely because it is ecofriendly and economical also. A wide variety of fungi, algae, and bacteria are now under study or used as biosorbents for different types of heavy metal remediation (Gadd, 1992; Volesky and Holan, 1995).

Thus the present study is designed to evaluate cytomodulatory effect of *Lactobacillus sporogenes* on ovary of arsenic-exposed mice.

Materials and Methods:-

Arsenic

In the present study Sodium Arsenate (Merk, Mumbai) was used for the experiment.

Microbes Used

Lactobacillus sporogenes was used as antidote procured from Synzyme Pvt Ltd Uttarakhand.

Experimental model

Female Swiss albino mice (*Mus musculus*) weighing 30±2gm were selected as an experimental model in the present study. All experimental procedures were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Ethical approval was obtained from Institutional Animal Ethics Committee of the institute.

Methodology:-

Chronic Toxicity Study: Selected pathogen-free mice were sorted and sodium arsenate was administered at 5 mg/kg body weight dose for 4 and 8 weeks by Gavage method. Sacrifices were done at the end of 4^{th} week and 8^{th} week of Sodium arsenate administration in each group.

Bioremediation: Sodium arsenate administration 5 mg/kg b.wt for 4 weeks was followed by the administration of *Lactobacillus sporogenes* for 8 weeks at 15 million spores/kg body weight. Animals were sacrificed on 4^{th} weeks and 8^{th} weeks of microbial administration.

Histopathological Studies: Mice were sacrificed from each group for histological analysis. The ovary tissue was dissected out and washed three times in isotonic saline (0.85 w/v %), fixed in neutral formalin solution and was processed. Slides were stained with Hematoxylin-Eosin (H & E) stains and examined under light microscope.

Hormonal Assessment: Blood was collected by orbital puncture and centrifuged to separate the serum to carry out estrogen analysis through ELISA reader.

Results:-

In control group, estrogen level was 22.33± 3.528 pg/ml. In arsenic four weeks and eight weeks administered group it was 254.0 ± 13.58 pg/ml and 606.0 ± 22.72 pg/ml. In arsenic eight weeks followed by four weeks and eight weeks administered group it was 148.3 ± 20.34 pg/ml and 31.67 ± 2.404 pg/ml respectively (Table-1, Graph-1)

Ovary of control mice shows normal mature graffian follicle. Ova were distinct. Graffian follicles were also normal. Germinal epithelium was normal in structure. Zona pellucid, theca externa and theca interna cells were also normal in structure (Figure -1).

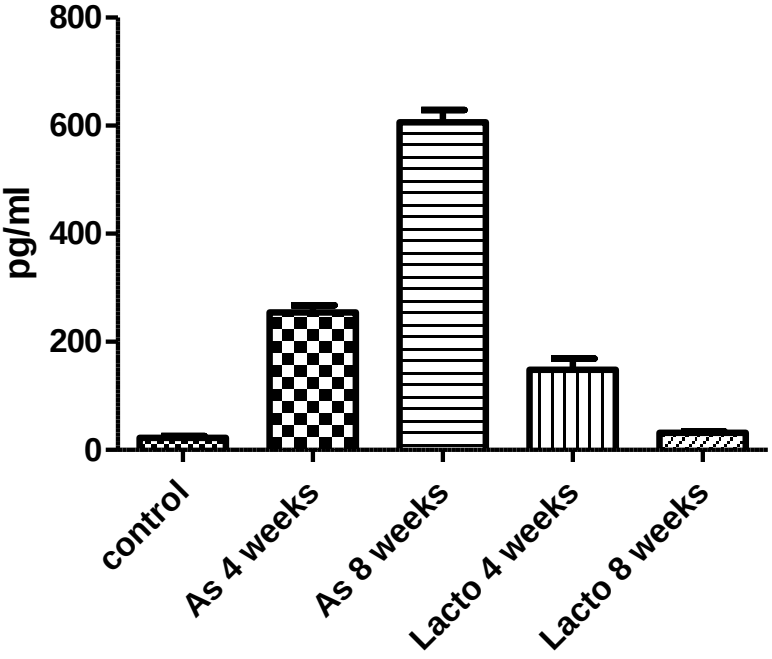
Ovary of Arsenic four weeks administered mice shows degenerated mature graffian follicle. Ovum degenerated to the greater extent. Different stages of follicular were observed. Clustered nuclei were observed in germinal epithelium. Many vacuolated spaces were observed in the ovarian medulla (Figure -2). Ovary of Arsenic four weeks administered mice shows degenerated corpus luteum. Ovum degenerated in different stages of follicle. Clustered nuclei were observed in germinal epithelium. Many vacuolated spaces were observed in ovarian cortex and medulla (Figure -3). Ovary of Arsenic eight weeks administered mice shows degenerated granulose cells. Mature graffian follicles were devoid of the ovum. Clustered nuclei were observed in germinal epithelium. Many vacuolated spaces were observed in the ovarian medulla (Figure -4). Ovary of Arsenic eight weeks administered mice shows degenerated granulose cells. Ovum was completely degenerated. Clustered nuclei were observed in granullosa cells. Many vacuolated spaces were observed in theca interna (Figure -5).

Ovary of Arsenic eight weeks administered mice followed by four weeks administration of lactobacillus shows restoration in corpus luteum. The different follicular structure was also restored. The estoration was observed in the germinal epithelium (Figure -6). Ovary of Arsenic eight weeks administered mice followed by four weeks administration of lactobacillus shows restoration in theca externa cells. Ovum was also restored. Corpus luteum was also restored effectively (Figure -7). Ovary of Arsenic eight weeks administered mice followed by eight weeks administration of lactobacillus shows restoration in corpus luteum. Both cytoplasmic and nuclear material of corpus luteum was restored effectively (Figure -8). Figure-9: Ovary of Arsenic eight weeks administered mice followed by eight weeks administration of lactobacillus shows restoration mature graffian follicles. Ovum was restored like the normal one. Granulose cells and theca cells are normal in structure. Ovarian cortex was also restored.

Table -1: showing estrogen level in different group of mice

Si.No	Group	Number	Level (pg/ml)
1.	**Control**	**03**	**22.33 ± 3.528**
2.	**Arsenic 4 weeks**	**03**	**254.0 ± 13.58**
3.	**Arsenic 8 weeks**	**03**	**606.0 ± 22.72**
4.	**Arsenic 8 weeks and Lacto 4 weeks**	**03**	**148.3 ± 20.34**
5.	**Arsenic 8 weeks and Lacto 8 weeks**	**03**	**31.67 ± 2.404**

Graph-1: Estrogen level in different group of mice



Discussion:-

Occupational exposure to arsenic among workers in a glass plant results in five times increase in arsenic which leads to increased DNA damage in leukocytes (Vuyyuri et al 2006). Genotoxic effects of sodium arsenite through the generation of reactive oxygen species were reported with the formation of micronuclei in the polychromatic erythrocytes in the bone marrow cells of Wistar rats (Balakumar et al, 2010). Portal tract fibrosis was reported in the liver of arsenic-exposed group (Mazumder et al, 2005). The urinary system is a more sensitive target for DMA than for MMA (Cohen et al, 2001). The evaluation of reproductive activity included a mating index; a fertility index and the precoital interval index were studied in details in arsenic-exposed animals (Holson et al, 1999).

Excess androgen production and relatively insufficient estradiol are major traits for poly cystic ovarian syndrome patients and essential for follicle development (Lebbe and Woodruff, 2013). These hormonal changes in the polycystic ovarian syndrome are likely associated with dysbiosis of gut microbiota. Microbiota such as Lactobacillus was used for the treatments of poly cystic ovarian syndrome in rats. Administration of probiotics such as Lactobacillus is an attractive concept in combating various diseases. L rhamnosus GR-1 attenuated lipopolysaccharide induced inflammation in pregnant CD-1 mice (Yang et al, 2014). Ingestion of probiotic lactic acid bacteria possibly would be a more natural method to decrease serum cholesterol concentrations in humans (McNamara et al, 1989). Cholesterol was the precursor for the formation of estrogen. *Lactobacillus* maintains normal cholesterol level in serum which causes restoration in the normal level of estrogen in arsenic-exposed group of mice.

Lactobacillus plays an important role in the maintenance of human health by stimulating the natural immunity and contributing to the balance of microbiota (McFarland, 2000). A previous study showed that postmenopausal women with a more diverse gut microbiome exhibited elevated urinary estrogens and estrogen metabolites (Fuhrman et al, 2014). Dysbiosis of gut microbiota has been implicated in many disease states, including diabetes, obesity and cardiovascular disease (Wang et al, 2011; Moran and Shanahan, 2014). Recently, a novel concept of "microgenderome" related to the potential bidirectional interaction roles between the sex hormones and gut microbiota has emerged (Flak et al, 2013). It has been reported that microbes of male and female animals diverged at the time of puberty, which affects sex hormone levels and exerts specific influences on microbiota composition of the organism. Presence of gut microbiota increased the testosterone level in female mice but decreased its level in male mice. Thus, the commensal gut microbiota also had effects on the production of male sex hormone (Markle et

al, 2013). *Lactobacillus* causes restoration of ova and ovarian follicles. Germinal epithelium was also restored to the greater extent with restored corpus luteum.

It is concluded from the entire study that Lactobacillus causes effective restoration in estrogen level. It also maintains germinal epithelium, follicular stages, corpus leuteum and ova in the ovary of mice. This indicates that Lactobacillus maintains normal female fertility in mice.

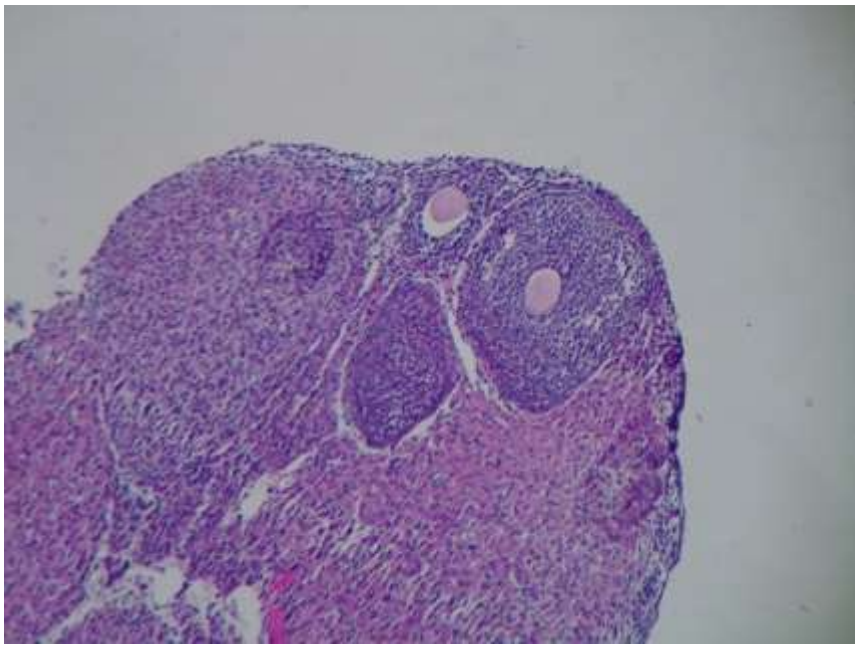


Figure1:- ovary of control mice shows normal mature graffian follicle. Ova was distinct. Graffian follicles were also normal. Germinal epithelium was normal in structure. Zona pellucid, theca externa and theca interna cells were also normal in structure. (500X).

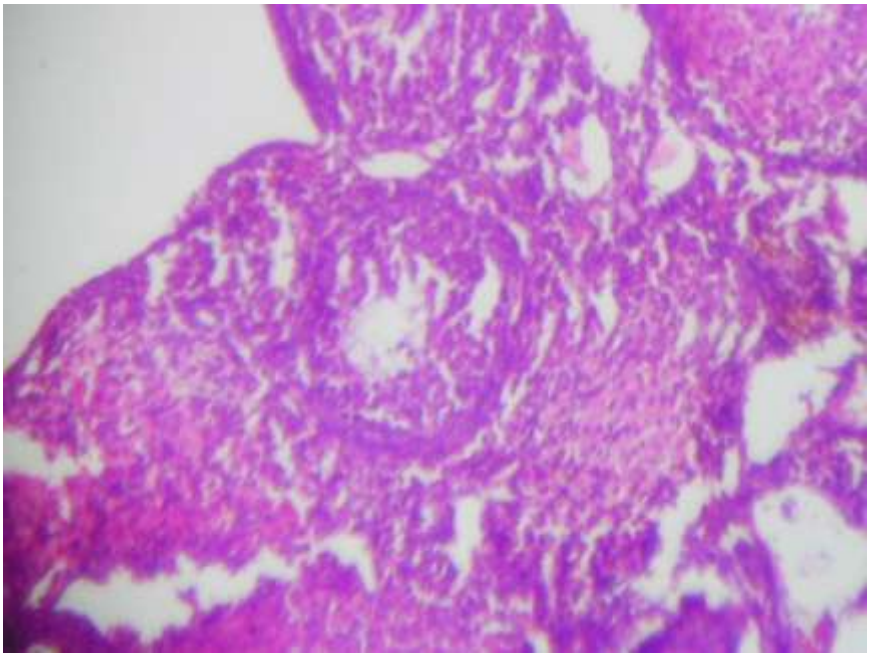


Figure 2:- ovary of Arsenic four weeks administered mice shows degenerated mature graffian follicle. Ovum degenerated to the greater extent. Different stages of follicular were observed. Clustered nuclei were observed in germinal epithelium. Many vacuolated spaces were observed in the ovarian medulla. (400X)

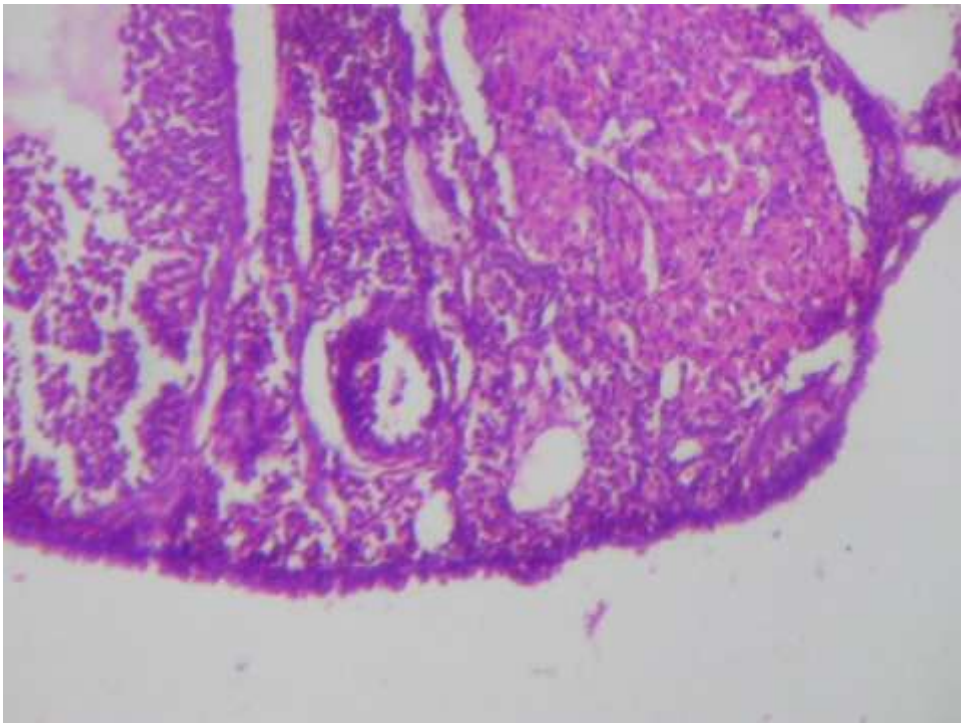


Figure 3:- ovary of Arsenic four weeks administered mice shows degenerated corpus luteum. Ovum degenerated in different stages of follicle. Clustered nuclei were observed in germinal epithelium. Many vacuolated spaces were observed in the ovarian cortex and medulla. (300 X).

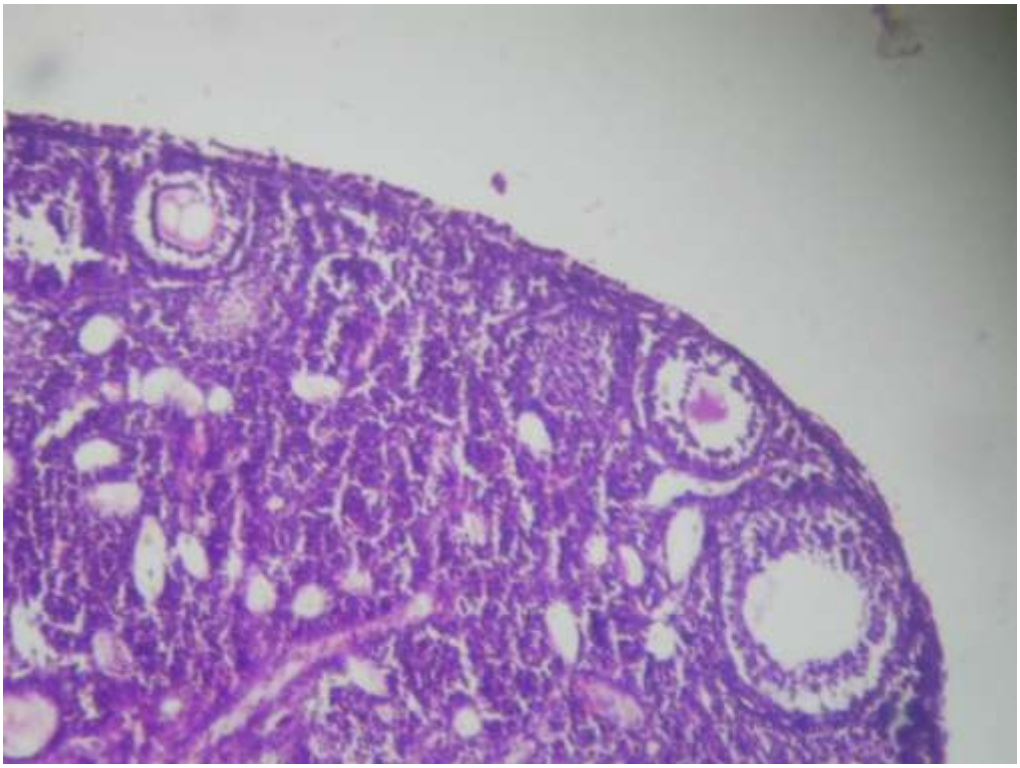


Figure 4:- ovary of Arsenic eight weeks administered mice shows degenerated granulose cells. Mature graffian follicles were devoid of the ovum. Clustered nuclei were observed in germinal epithelium. Many vacuolated spaces were observed in the ovarian medulla. (300X)

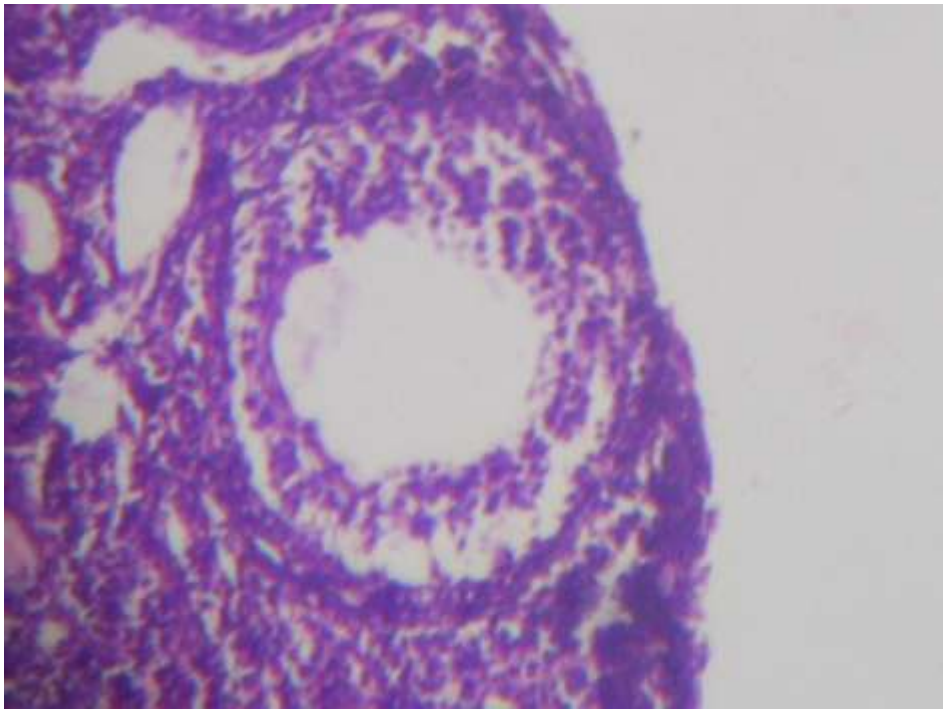


Figure 5:- ovary of Arsenic eight weeks administered mice shows degenerated granulose cells. Ovum was completely degenerated. Clustered nuclei were observed in granulosa cells. Many vacuolated spaces were observed in theca interna. (600X)

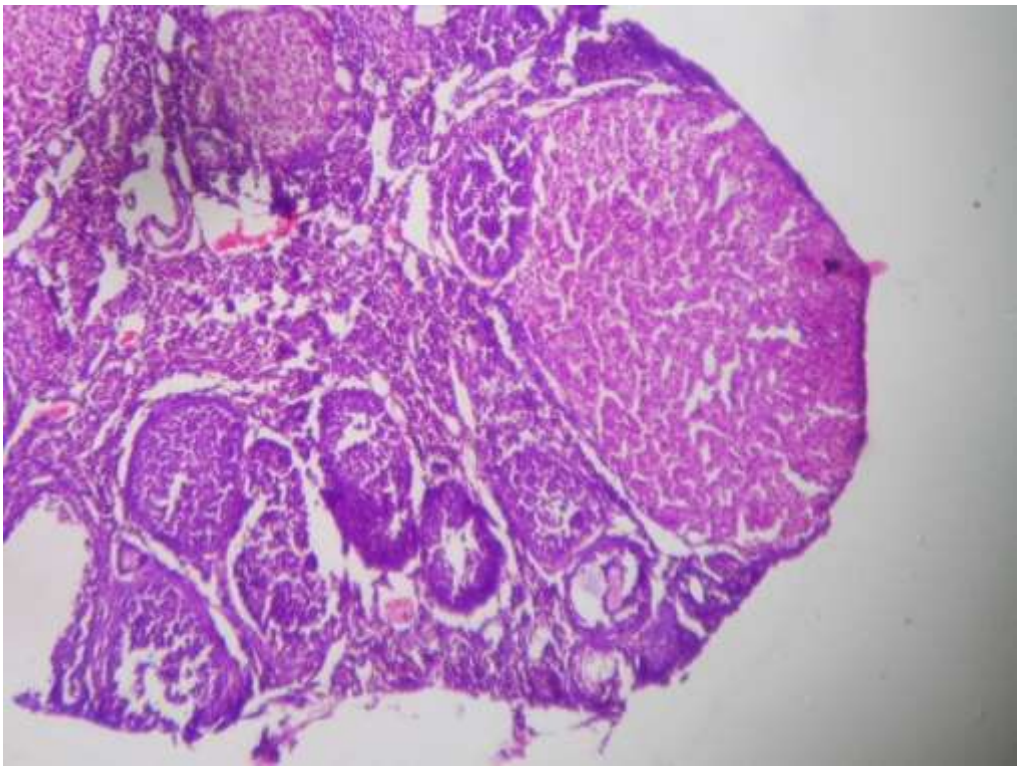


Figure 6:- Ovary of Arsenic eight weeks administered mice followed by four weeks administration of lactobacillus shows restoration in corpus luteum. The different follicular structure was also restored. The restoration was observed in germinal epithelium. (400 X)

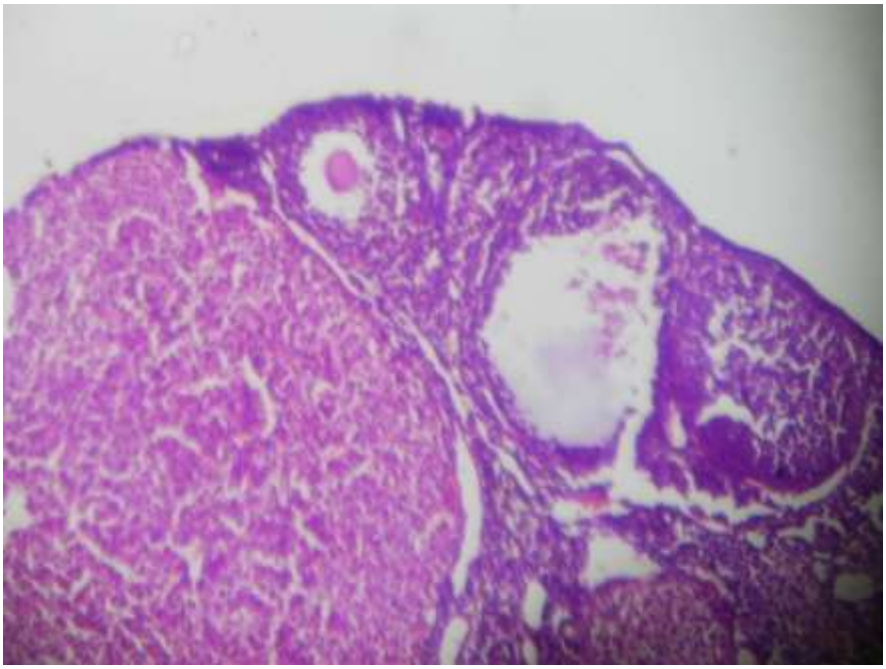


Figure 7:- Ovary of Arsenic eight weeks administered mice followed by four weeks administration of lactobacillus shows restoration in theca externa cells. Ovum was also restored. Corpus luteum was also restored effectively. (400 X)

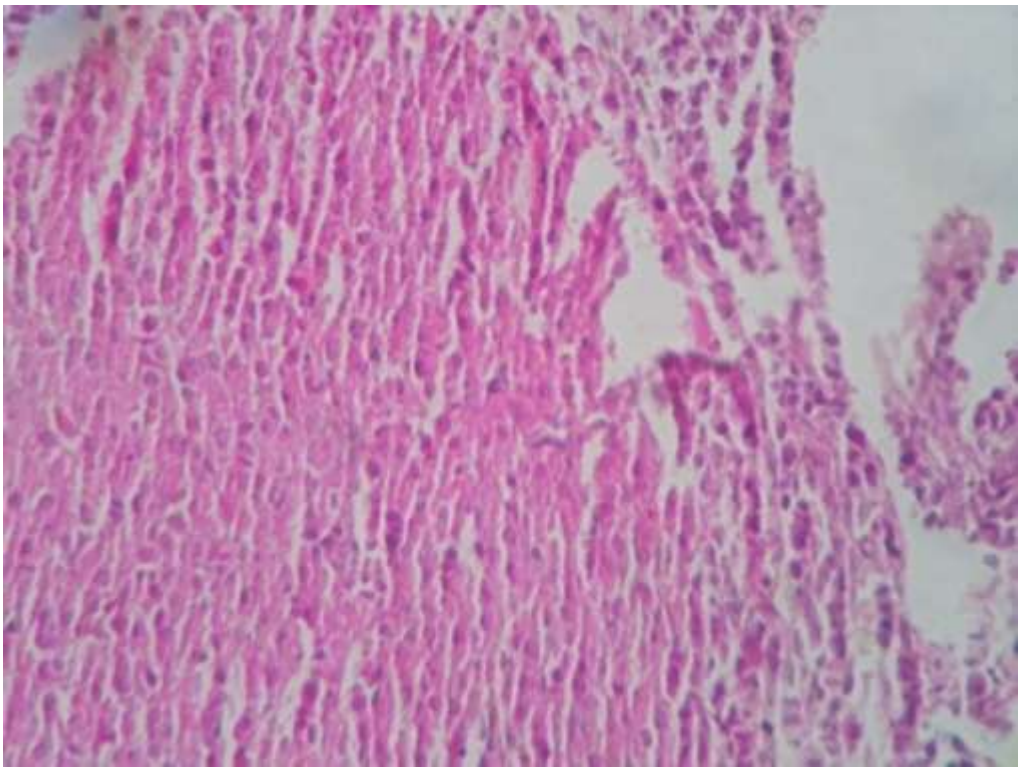


Figure 8:- Ovary of Arsenic eight weeks administered mice followed by eight weeks administration of lactobacillus shows restoration in corpus luteum. Both cytoplasmic and nuclear material of corpus luteum was restored effectively. (800 X)

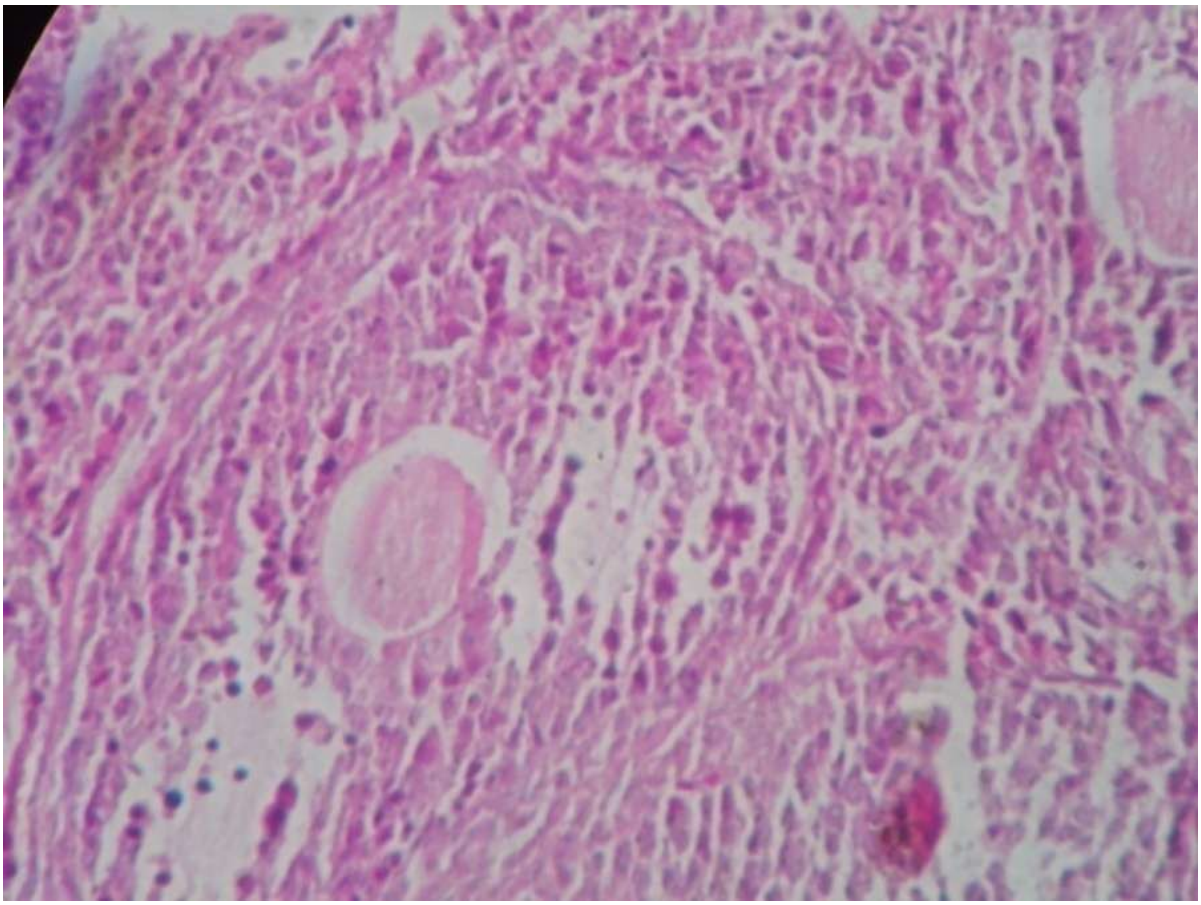


Figure 9:- Ovary of Arsenic eight weeks administered mice followed by eight weeks administration of lactobacillus shows restoration mature graffian follicles. Ovum was restored like the normal one. Granulose cells and theca cells are normal in structure. Ovarian cortex was also restored. (800 X)

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