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Amelioration of Lead induced toxicity on rat ovary with *Linum usitatissimum* (flaxseed) and *Emblica officinalis* (Amla)

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Abstract
Lead, as one of the environmental pollutants, can threat the lives of animals and human beings in many ways; especially during developing stages. Among heavy metals lead is one of the most toxic metals that induce a wide range of behavioral, biochemical, physiological and reproductive dysfunctions in humans and animals. The rationale of current study was to observe the effects of lead acetate on ovary and the protective role of *Linum usitatissimum* (Flaxseed) and *Emblica officinalis* (Amla) in female wistar rats. 108 female adult wistar albino rats were randomly assigned into six groups consisting of 18 rats in each, where Group I served as control and they received distilled water. Group II provided with lead acetate @ 60 mg/ kg b.wt., Group III had *Emblica officinalis* @ 100 mg/ rat/ day, Group IV was fed with *Linum usitatissimum* @ 300 mg/ kg b.wt, Group V was given lead acetate @ 60 mg/ kg b.wt + *Emblica officinalis* @ 100 mg/ rat/ day and Group VI received lead acetate @ 60 mg/ kg b.wt + *Linum usitatissimum* @ 300 mg/ kg b.wt. orally for 45 days. From each group six rats were sacrificed at fourteen days intervals. Blood collected at each sacrifice was allowed to clot for separation of serum and later used for estimation of serum Progesterone and estrogen. Ovary was collected and preserved in neutral buffer Formalin (NBF) for histopathological study. Serum Progesterone (P) and Estrogen (E2) concentration was significantly ($P< 0.05$) decreased in lead treated group. Light microscopic study of ovary showed severe degenerative changes and Immunohistochemistry with BAX markers in ovary revealed intense immunoreactivity in lead treated rats. The levels of all above parameters were significantly improved in the ameliorated group (Group V and VI). The observations were made in the study indicated that treatment of Amla and Flaxseed to rats concurrently with the lead was shown to have ameliorating effect on different pathological manifestations and the ameliorating effect of Emblica was found to be relatively better than that of Flaxseed except in the levels of sex hormones in which the latter showed better effect (increased the levels).

Keywords: Lead, flaxseed, amla, progesterone, estrogen, immunohistochemistry

Introduction
Lead (Pb), the most abundant toxic heavy metal in the environment, has been consequently increased due to the extensive commercial use of lead (Pb) from prehistoric times despite its recognized hazards and it is one of the most toxic metal that induce a wide range of behavioural, biochemical, physiological and reproductive dysfunctions in humans and animals (Pokras and Kneeland, 2009) [22]. In veterinary medicine lead intoxication is one of the most frequently encountered poisoning world-wide and has been recorded in all domestic and several zoo species.

Lead is toxic for virtually all organs of the body and has significant debilitating effects on the nervous, renal, hepatic, reproductive and hematopoietic systems (Patrick, 2006) [21]. Inhalation and ingestion are the two most common routes of entry of lead into the body. Lead exhibits toxic effects by various mechanisms, including alteration of enzymatic function, generation of oxidative stress, interfering with the action of essential cations, mainly zinc, calcium and iron, disruption of the integrity of cellular membrane and organelles and through modification of cell signalling (Katzung *et al*., 2010) [17].

From the view point of reproduction, lead is known to cause a number of adverse consequences in both human and animal. Some studies suggested that low doses of lead affect reproduction and sexual development in men, women and small mammals either directly or indirectly (Junaid *et al*., 1997, Qureshi and Sharma, 2012) [16, 25]. However, systematic evaluation of female reproductive system comparing anatomical and functional parameters is missing.

In recent years, research work has thrown light on the use of plants as they have plenty of health benefits due to presence of wide variety of medicinal properties. The use of *Emblica officinalis* (Emblica) or amla has been reported in Indian traditional medicine and its modern applications are receiving wide spread attention day by day. Extract from Emblica is proven to be potent antioxidant, anti-inflammatory (Chang *et al*., 2013 and Pradyumna *et al*., 2013) [6, 23], anti-diabetic, chemoprotective agent (Golechha *et al*.,2012, Wiart, 2013) [11, 39]. Emblica is a rich source of tannins (Zhang *et al*., 2001) [41], polyphenols, flavones (Anila and Vijayalakshmi, 2002) [4] and some bioactive substances. Among them tannaoids are the active principles present in amla having vitamin C like properties (Ghosal *et al*., 1996) [10] which act as anti-inflammatory, potent antioxidant and anti-mutagenic agents. Different studies suggest that antioxidants have an important role in abating some hazards of lead (Shrama *et al*., 2011) [30].
Linum usitatissium (Flaxseeds) or linseed is one of the most significant sources of plant lignans. Flaxseed lignans are rich source of phytoestrogen, which have estrogen-like effect (Nesbitt *et al*., 1997) [19]. Flaxseed claimed to be valuable in a wide spectrum of diseases. It has been observed that flaxseed has antioxidant (Rhee and Brunt, 2011) [23], cardioprotective (Zanwar *et al*., 2011) [40], chemo protective (Tham *et al*., 1998) [34], hepato protective (Abdou and Newairy, 2006) and anti-inflamatory (Troina *et al*., 2010) [35] properties. Due to its several beneficial effects on health daily consumption of flaxseed and its modern application in medicine are receiving widespread attention in recent days. Several authors have tried different ameliorating agents like vitamin- C, E, garlic extract, selenium etc. against different heavy metal toxicity. Keeping in view in this present study Emblica and Flaxseed was used as an ameliorating agent to find out the protective role of these plants against Lead toxicity.

Materials and Methods

Procurement of experimental animals, aqueous extract of *Emblica officinalis* and lead acetate

Female Wistar albino rats with body weight around 150 to 200 gms were procured from Sri Venkateswara Agencies, Bangalore and after one week of acclimatization the rats were randomly divided into six groups and housed in standard polypropylene rat cages (three rats/cage). They were maintained at 25 °C ± 10 °C and a 12:12 hour interval light and dark cycle during the 45 days of experimental period by maintaining standard laboratory hygienic conditions with *ad libitum* supply of feed and water.
The Lead acetate was procured from the Qualigens Fine Chemicals Company, product code No.27645 with 97% purity. Aqueous extract of Amla (*Emblica officinalis*) with product code C/SVU/EMOF-01 was procured from Chemiloids Company, Vijayawada, India.

Ethical matters

The ethics governing the use and conduct of experiments on laboratory animals were strictly observed and the experimental protocol was approved by Institutional Animal Ethical Committee (IAEC), Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India (Reference number-
281/go/ReBi/S/2000/CPCSEA/CVSC/TPTY/018/VPP/2016-17. Institutional Animal Ethical Committee (IAEC), Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, is affiliated under The Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA, India) with the Registration number-281/go/ReBi/S/2000/CPCSEA.

Preparation of aqueous methanolic extract of *Linum usitatissimum* (Flaxseed)

Seeds of *Linum usitatissimum* (Flax seeds) were procured from a local herbal shop. After drying in shade, seeds were ground into powder form, which then used for the preparation of aqueous methanolic extract. Flaxseeds (800g) were ground using a grinder to attain a fine powder form. The powder form of flaxseeds was defatted by blending with hexane (1:6 w/v, 12 h) in room temperature. The defatted flaxseeds powder was air dried for around 12 hours. 200 grams of this defatted powder was again blended with 1.2 Litres complex solution of ethanol and water (7:3 v/v) for 24 hours at ambient temperature (25 °C). The extract was filtered into flask, and then filtered product was concentrated at 50 °C using a Rotary evaporator (Buch, Model 462, Germany) @ 90 rpm. Light yellow color syrup of flaxseed lignans extract was obtained (Zhang *et al*., 2007) [42].

Experimental design

108 no. of female adult wistar albino rats were assigned to 6 groups randomly with 18 rats in each group. Group I served as vehicle control and they received distilled water, whereas animals in Group II, III, IV, V and VI received lead acetate @ 60 mg/ kg b.wt., *Emblica officinalis* @ 100 mg/ rat/ day, *Linum usitatissimum* @ 300 mg/ kg b.wt, lead acetate @ 60 mg/ kg b.wt + *Emblica officinalis* @ 100 mg/ rat/ day, lead acetate @ 60 mg/ kg b.wt + *Linum usitatissimum* @ 300 mg/ kg b.wt. respectively for 45 days. From each group six rats were sacrificed at fourteen days intervals.

Hormonal assay and Histopathology

Blood was collected at each sacrifice from all the group and allowed to clot for seperation of serum. Serum stored at 4°c until use for the valuation of estrogen and progesterone by ELISA method by using kits obtained from Omega Diagnostics, Alva (Scotland) and Estrogen (E2) using Calbiotech, Spring Valley (U. S. A). A detailed post-mortem examination was carried out on the sacrificed rats of all the trial groups. The gross changes were documented and ovary was collected and well-preserved in 10% NBF for histopathological studies, fixed tissues were processed by standard paraffin embedding technique. 5-6 microns thick tissue sections were cut and section were stained with Haematoxylin and Eosin stain (H&E) (Culling 1974) [8].

Immunohistochemistry

To assess the apoptosis in ovary Bax marker was used in both treated and non-treated groups. The primary and secondary antibodies for immunohistochemical studies were obtained from Biogenex Company. Immunohistochemistry was performed on formalin fixed paraffin embedded tissue. Paraffin embedded tissue sections were cut at thickness of 3-4 microns and kept at 56 °C for 2 hours. Deparaffinised via xylene for 15 min, 2 changes and then dips in alcohol to remove xylene. Placed the slide in running tap water for 10 min and rinsed in distilled water for 5-10 min. Then slides were kept in citrate buffer solution for 20 min (15 min in medium power and 10 min in high power in microwave oven). Cooled to the room temperature, placed in distilled water for 5 min and kept the slide in PBS for 5 minutes. The

slides were placed in humid chamber in the peroxidase block solution for 30 min (for blocking the endogenous peroxidase). Placed the slide in PBS for 5 minutes X 3 changes. Added super enhancer solution and the slides were placed in PBS for 5 minutes X 3 changes. Added secondary antibody with HRP (BAX) for 30 min. Placed in PBS for 5 minutes X 3 changes. DAB colouring reagent was prepared by mixing one drop dab with 1 ml substrate. The colouring reagent were added in tissue section and kept for 5 to 8 min. Washed in PBS for 2 min to stop the reaction, and in distilled water for 2 min. Counter stained with Harris haematoxylin for 1 min. washed under tap water for 5 min. Dried the slide and mounted in DPX. The results were analyzed statistically by performing one way ANOVA (Snedecor and Cochron, 1994) [31].

Results and Discussion

Clinical sign: During the present study, clinical signs were seen in rats of lead acetate treated group from 5th week onwards which include reduced feed intake, reduction in growth rate, anxiety and ruffled hair. Continuous scratching of face and hyper irritability like nervous symptoms were also noticed. The similar clinical sign were observed by Haque *et al*. (2006) [13]. The clinical signs in lead fed group rats might be due to penetration of lead in to blood brain-barrier and inhibition of transport protein (transferring) synthesis (Batra *et al*., 1998 and Hunuman *et al*., 1999) [14]. Group III (Emblica treated) and Group IV (Flaxseed treated) were apparently normal without any clinical signs and there was no mortality in rats of any of the treated groups during the whole experimental period.

Hormonal assay

The mean serum progesterone (P) concentration from Group I to VI were 23.90, 11.83, 25.13, 26.43, 21.83 and 23.40 (ng /ml) respectively and presented in **Table 1.** and the mean serum estrogen (E_2) concentration in Group I to VI were 1248.66, 214.2, 1284.5, 1320.66, 1155.7 and 1230.13 (pg/ mL) respectively and presented in **Table 2.** Above findings indicate that significant ($P< 0.05$) decrease in mean serum progesterone (P) and estrogen (E_2) concentration in lead acetate treated rats (Group II) when compared to the control rats (Group I). Similar observations were reported by Abdou and Newairy (2006) [1] and Waseem and Rehman (2015) [37]. Decrease in serum progesterone and Estrogen level might be due to down regulation of P and E_2 by activating their metabolizing enzymes, 5β reductase and 17β-HSD, respectively (Abdou and Newairy, 2006) [1].

Emblica ameliorated group (Group V) showed significant increase in mean serum Progesterone and estrogen concentrations and this might be due to ascorbic acid present in Emblica which play an important role in the activation of adenyl cyclase and inhibition of phosphodiesterase (PDE) resulting in higher c-AMP levels that prevents the decline in serum P and E2 concentration (Pasternak, 1979) [20]. Significant increase in mean serum P and E2 concentration was observed in Flaxseed ameliorated rats (Group VI) compared to the lead treated group. Lignans present in flaxseed normalized the activity of both 5β- reductase and 17β-hydroxysteroid dehy- drogenase (17β-HSD) (Abdou and Newairy, 2006) [1] and also involved in the balance between estradiol and estrone (Gunnarsson *et al*., 2001). Thus justify the improvement of hormone levels in group-VI.

There was no significant alteration in Serum progesterone and estrogen level was observed in Emblica (Group III) and Flaxseed (Group IV) treated rats.

Table 1: Mean serum progesterone (ng/ mL) in rats of different experimental groups.

Weeks	Group I	Group II	Group III	Group IV	Group V	Group VI
2	22.7	15.8	23.5	24.9	19.7	20.5
4	23.5	11.3	25.2	25.6	22.3	23.8
6	25.5	8.4	26.7	28.8	23.5	25.9
Mean ±S.E	23.9 ±0.83 [a]	11.83 ± 2.15 [b]	25.13 ± .92 [a]	26.43 ± 1.2 [a]	21.83 ± 1.12 [a]	23.4 ±1.57 [a]

Mean values with different superscripts differ significantly ($P< 0.05$), ANOVA
S.E- Standard Error

Table 2: Mean serum estrogen (pg/ mL) in rats of different experimental groups

Weeks	Group I	Group II	Group III	Group IV	Group V	Group VI
2	1123.5	253.3	1251.2	1265.7	998.1	1123.4
4	1347.8	218.7	1293.9	1326.8	1217.30	1279.1
6	1274.7	170.6	1308.4	1369.5	1251.7	1287.9
Mean ± S.E	1248 ± 66.04 [a]	214.2 ± 23.97 [b]	1284.5 ± 17.16 [a]	1320.66 ± 30.12 [a]	1155.70 ± 79.42 [a]	1230.13 ±53.42 [a]

Mean values with different superscripts differ significantly ($P < 0.05$), ANOVA
S.E- Standard Error

Gross and Histopathology

Ovaries of lead acetate treated rats (Group II) and ameliorated group (V and VI) were unchanged up to 4th week of experiment. By the end of 6th week of experiment, small and atrophied ovaries in Group II rats were evident whereas Group V and VI rats had almost near to normal ovaries. On microscopic examination, ovaries of Group II rats revealed congestion of blood vessels in medulla, degenerative changes in cortex, degenerated primordial, primary unilaminar and primary multilaminar follicles (Fig: 1) and secondary or antral follicle with mild to moderate disintegration of oocyte leaving space in primary follicle and mild degeneration of granulossa cell and hemorrhages in corpus luteum were observed by the end of 2nd week. Thickened tunica albuginea, severely degenerated granulosa cells, degenerated and loss of luteal cells in corpus luteum and the space is filled with fibroblasts, RBC and with MNCs infiltration (Fig: 2) and corpus luteum surrounded by proliferated fibrous tissue and also in cortex were noticed by the end of 4th week. In addition to the above changes, greater thickening of tunica albuginea with fibrous tissue proliferation, completely degenerated primordial and primary unilaminar and multilaminar follicles and secondary follicle, lysis of oocyte, cumulus oophorus and clumping of granulosa cells and found in antrum of secondary follicle, completely degenerated granulosa cells with complete disintegrated oocyte and its nucleus in secondary follicle (Fig:

3), hemorrhages and fibroblast proliferation between interstitial tissue in cortex, pockets of hemorrhages in corpus luteum, increased number of atretic follicles, cortical vacuolation beneath the tunica albuginea and plumpy interstitial cells with vacuolated cytoplasm. Significant reduction in the number of follicles at different developmental stages (Fig: 4) were more evident by the end of 6th week of experiment. These findings were gained support from earlier researchers Eugenia *et al*. (2009) [9] and Waseem *et al*. (2014) [38]. These above changes might be associated with lead induced oxidative stress includes the damaging effects on cell membranes, DNA and antioxidant defense systems of cells (Ahamed and Siddiqui, 2007) [3]. Lead also effect ovarian folliculogenesis and interference with endocrine, paracrine and biochemical pathways of ovary (Taupeau *et al*., 2001) [33] also justify the changes in ovarian histology.

Histopathology of ovaries of Group V rats was similar to that of Group II up to 2nd week. Later on, severity of lesions was decreased like mild degenerative changes in granulosa cells, focal loss of luteal cells and normal tunica albuginea were observed. Ovaries regained the structure near to its normal appearance and number of follicles of different stages were increased (Fig: 5) to some extent by the end of 6th week of experiment. This could be attributed to the improved protein synthesis or potent free radical scavenger property of vitamin C content of Emblica (Chinoy and Sharma, 1998) [7].

Histopathological examination of ovaries of Flaxseed ameliorated rats (Group VI) exhibited similar lesions with mild intensity up to 2nd week and by the end of experiment increase number of ovarian follicle at different developmental stages (Fig: 6) were evident and ovaries regained almost its near to normal appearance with restored architecture of granulosa cells of ovarian follicles and corpus luteum, normal tunica albuginea. Antioxidant properties of secoisolariciresinol diglucoside (SDG) present in flaxseed which also acts as a precursor of mammalian lignans called phytoestrogen (Prasad, 2004 and Adolphe *et al*., 2010) [24, 2] might be associated with the improvement in ovary of Group-VI rats.

There was no significant gross and microscopic alteration was observed in ovary of Emblica (Group III) and Flaxseed (Group IV) treated rats.

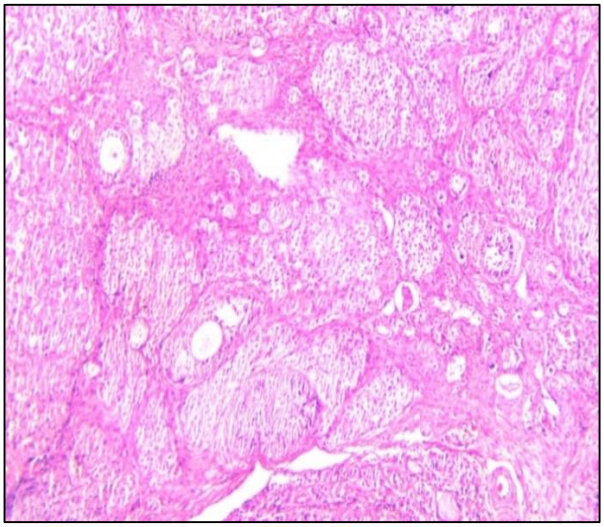

Fig 1: Ovary: Group II: Section showing degenerated primary unilaminar and multilaminar follicles. H & E: x 100

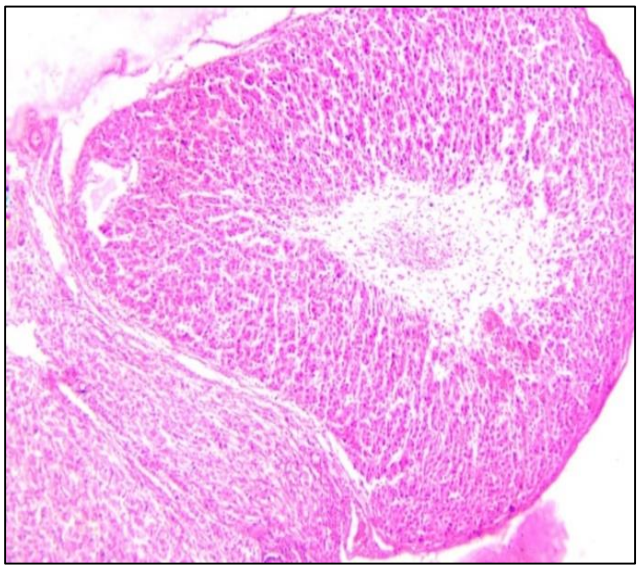

Fig 2: Ovary: Group II: Section showing loss of luteal cells in corpus luteum and the space is filled with fibroblasts, RBCs and infiltration of MNCs. H & E: x 100

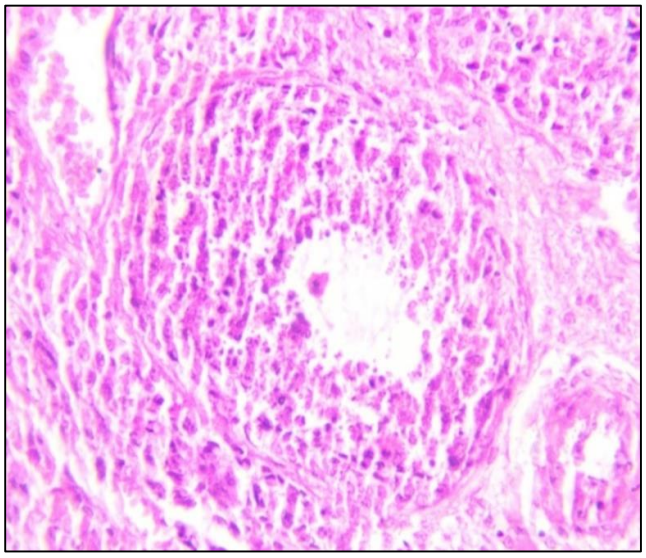

Fig 3: Ovary: Group II: Section showing severely degenerated granulosa cells with completely disintegrated Oocyte. H & E: x 400

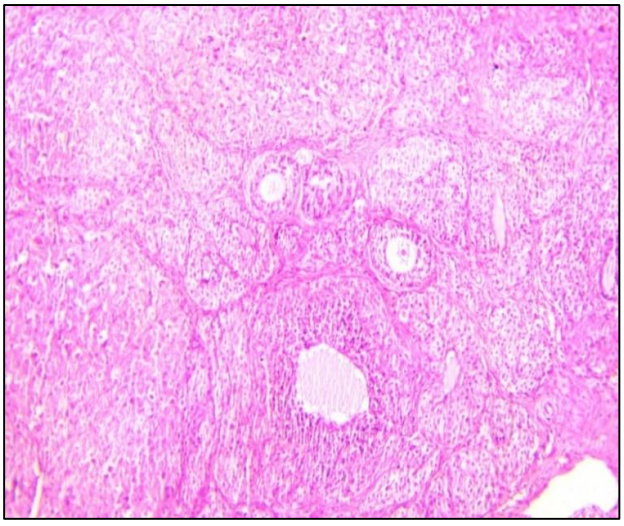

Fig 4: Ovary: Group II: Note reduced number of different stages of follicles. H & E: x 100

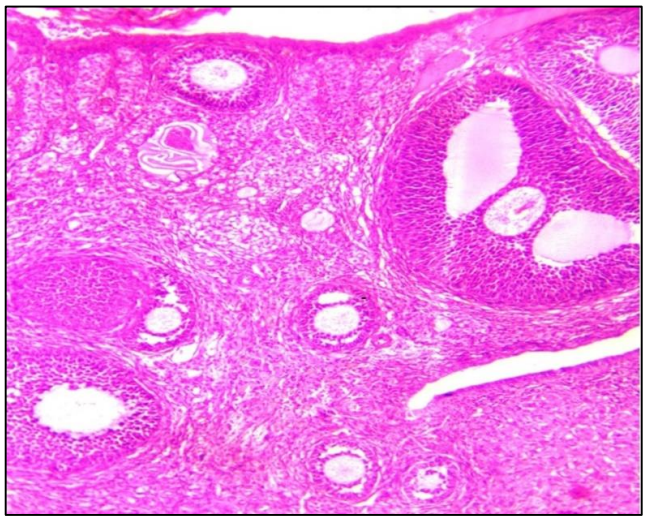

Fig 5: Ovary: Group V: Section showing restored different stages of follicles. H & E: x 100

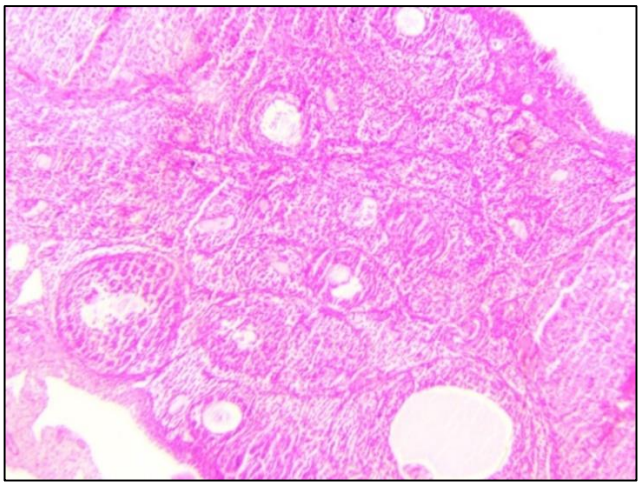

Fig 6: Ovary: Group VI: Section showing almost normal appearance. H & E: x 100

Immunohistochemistry

Immunohistochemistry was done using monoclonal antibodies against BAX antigens for the assessment of apoptotic changes in ovary. BAX antigen is well expressed during apoptosis and at the site where the intense brown colour develops indicates the presence of BAX antigen. Immunohistochemistry, control rats (Group I) showed minimal expression of BAX marker (Fig. 7) whereas the BAX antigen with higher intensity was detected in the granulosa cell of ovarian follicles, luteal cell of corpus luteum (Fig. 8, 9, 10) and also in interstitial tissue of lead treated rats (Group II). The similar observations were made by Wang *et al*. (2007) [36], Jin xu *et al*. (2008) [15], Sujatha *et al*. (2011) [32]. This is probably due to lead induced alteration in the cell respiration and inhibition of energy production. In addition lead induced oxidative damage and changes the expression of apoptosis relate protein (Jin xu *et al*., 2008) [15].

In Emblica ameliorated rats (Group V) decreased expression of BAX antigen was observed (Fig. 11) in ovarian tissue. This might be due to the protective role of vitamin C present in Emblica and thus blocks the apoptotic pathway (Serbecic and Beutelspacher, 2005 and Ramanathan *et al*., 2005) [29, 26]. Decreased expression of BAX antigen was observed in ovary of Flaxseed ameliorated rats (Group VI). This might be due to presence of Omega-3 fatty acid and SDG in flaxseed which exhibit antioxidant properties (Sekine *et al*., 2008) [28] and protect cells from oxidative stress induced cell damage. No significant alterations were noticed in Emblica (Group III) and Flaxseed (Group V) treated rats.

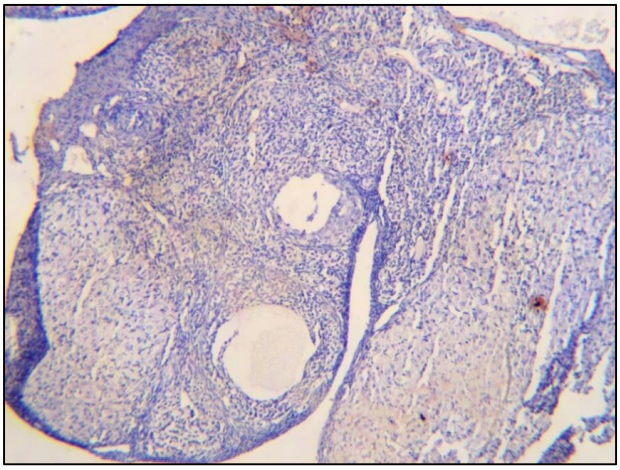

Fig 7: Ovary: Group I: Immunohistochemistry: Section showing minimal immunoreactivity in corticular area. BAX: x 40

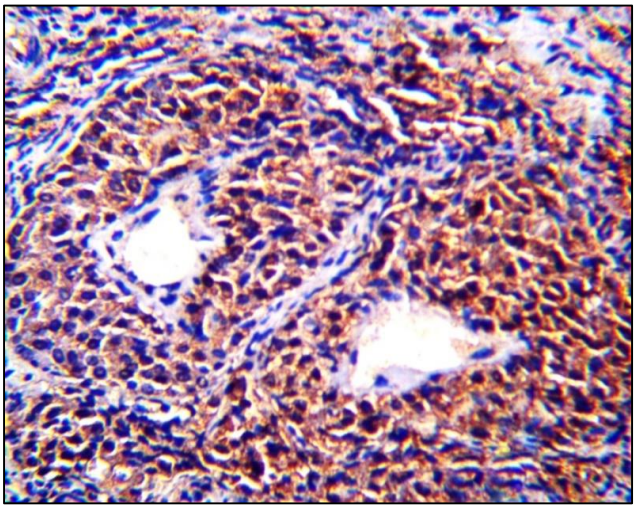

Fig 8: Ovary: Group II: Immunohistochemistry: Section showing moderate to severe immunoreactivity in granulosa cells of developing follicles. BAX: x 400

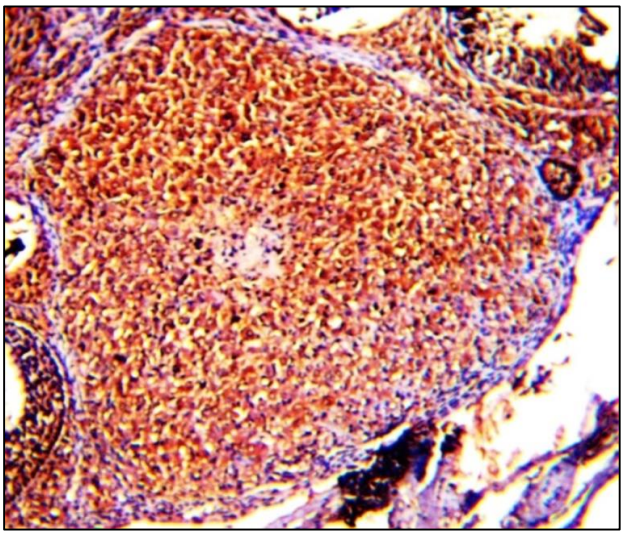

Fig 9: Ovary: Group II: Immunohistochemistry: Section showing severe immunoreactivity in cells of corpus luteum. BAX: x 100

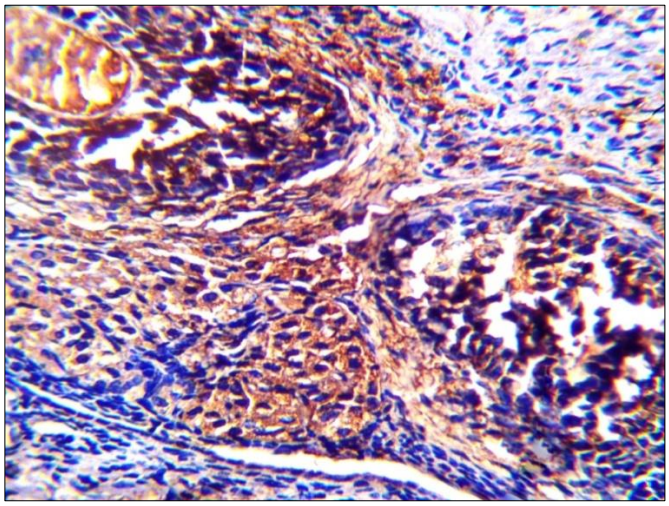

Fig 10: Ovary: Group II: Immunohistochemistry: Section showing moderate to severe immunoreactivity in granulosa cells of developing follicles and theca cells. BAX: x 400

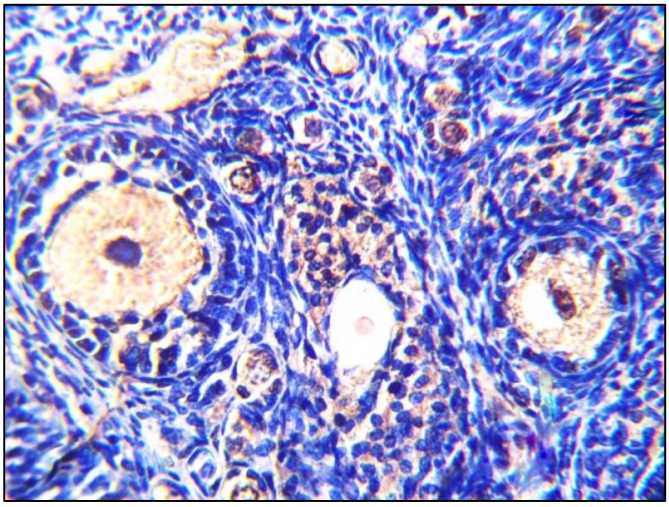

Fig 11: Ovary: Group V: Immunohistochemistry: Section showing minimal immunoreactivity in primordial germ cells and primary follicles. BAX: x 400

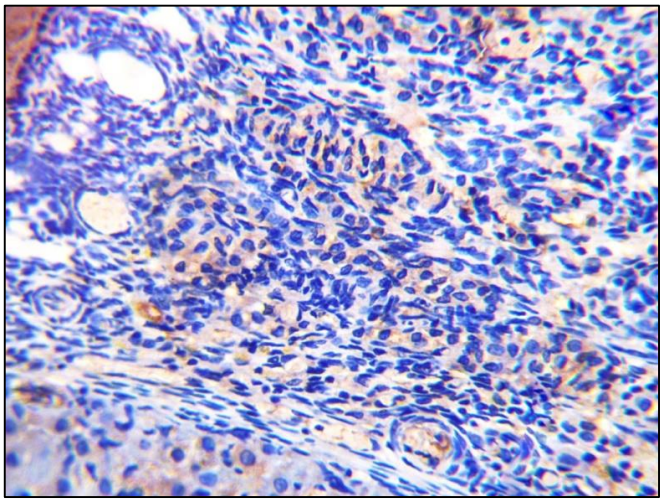

Fig 12: Ovary: Group VI: Immunohistochemistry: Note medullary area of the ovary showing minimal immunoreactivity. Left below-portion of follicles showing minimal reactivity in granulosa cells. BAX: x 100

Conclusion

The findings from this study implies that lead (Pb) is having adverse effect on ovary and caused various pathological alterations and supplementation of Emblica and Flaxseed to rats in combination with the lead was shown to have ameliorating effect on different pathological manifestations. Present study revealed Emblica is relatively better than that of Flaxseed in ameliorating the different degenerative changes occurred in ovary except in the cocentration of sex hormones in which flaxseed showed better result (increased the sex hormone levels) as flaxseed contain high source of phytoestrogen. So, from the present investigation point of view and results of this investigation infer that Emblica and Flaxseed play a preventive role in reducing lead poisoning. Use of this herbal agent in veterinary and human medicine can be useful in heavy metal toxicity as a supportive therapy to minimize the harmful and toxic effects of different heavy metals like lead.

References

1. Abdou HM, Newairy AA. Hepatic and Reproductive Toxicity of Lead in Female Rats and Attenuation by Flaxseed Lignans. J M R I 2006;27(4):295-302.
2. Adolphe JL, Whiting SJ, Juurlink BHJ, Thorpe LU, Alcorn J. Health effects with consumption of the flax lignan secoisolariciresinol diglucoside. Br J Nutr 2010;103:929-938.
3. Ahamed M, Siddiqui MK. Low level lead exposure and oxidative stress: current opinions. Clin Chim Acta 2007;383(1-2):57-64.
4. Anila L, Vijayalakshmi NR. Flavinoids from *Emblica officinalis* and *Mangifera indica* effectiveness for dyslipidemia. J Ethnopharmacol 2002;79(1):81-87.
5. Batra N, Nehru B, Bansal MP. The effect of Zinc Supplementation on the effects of lead on the rat testis. Reprod Toxicol 1998;12:535-540.
6. Chang KC, Laffin B, Ponder J, Enzosly A, Nemeth J, Barbera LD *et al*. Beta-glucogallin reduces the expression of lipopolysaccharide induced inflammatory markers by inhibition of aldose reductase inmurine macrophages and ocular tissues. Chem Biol Interact 2013;202(1, 3):283-287.
7. Chinoy NJ, Sharma AK. Amelioration of fluoride toxicity by vitamin E and D in reproductive function of male mice. Fluoride 1998;31:203-216.
8. Culling CFA. Hand Book of Histopathological and Histochemical Techniques (Including Museum Techniques). Edn 3. Butterworth-Heinemann, London, 1974, 361.
9. Eugenia D, Alexandra T, Diana A, Cristina RT. The consequences of in utero exposure to lead acetate on exposure and integrity biomarkers of reproductive system in female rats at sexual maturity. Lucrari Ştiinłifice Med Vet 2009;42(2):295-300.
10. Ghosal S, Tripathi VK, Chauhan S. Active constituents of *Emblica officinalis*: Part I- The chemistry and antioxidant effects of two new hydrolysable tannins, Emblicanin –A and B. Indian J Chem 1996;35:941-948.
11. Golechha M, Bhatia J, Arya DS. Studies on effects of *Emblica Officinalis* (Amla) on oxidative stress and cholinergic function in scopolamine induced amnesia in mice. J Environ Biol 2012;33(1):95-100.
12. Gunnarsson C, Olsson B, Stal O. Abnormal expression of 17u-HSD in breast cancer predicts late recurrence. Cancer Res 2001;61:8448-51.
13. Haque MM, Awal MA, Mostofa M, Sikder MH, Hossain

MA. Effects of calcium carbonate, potassium Iodide and Zinc sulphate in lead induced toxicities in rat model. Bangladesh J Vet Med 2006;4(2):123-127.
14. Hnuman YT, Da KC, Xue B. Experimental and Clinical study to remove lead and to prevent lead damage of kidney with salt of high dose. Korean J Environ Qual 1999;24:539-42.
15. Jin Xu, Ling-Jun Lian Chen, Wu Xiao-Feng, Wang Wen-Yufu, Li-hong XU. Lead induces Oxidative Stress, DNA damage and alteration of P53, BAX and Bcl2 expression in mice. Food Chem Toxicol 2008;46(5):1488-1494.
16. Junaid M, Chowdhuri DK, Narayan R, Saxena DK. Lead-induced changes in ovarian follicular development and maturation in mice. J Toxicol Enviro Health 1997;50:31-40.
17. Katzung BG, Masters SB, Trevor AJ. Basic and Clinical Pharmacology. Edn 11, McGraw-Hill, New York 2010, 999-1117.
18. Lyn Patrick ND. Lead toxicity part II: The role of free radical damage and the use of antioxidant in the pathology and treatment of lead toxicity (Review). Altern Med Rev 2006;11:114-27.
19. Nesbitt PD, Thompson LU. Lignans in homemade and commercial products containing flaxseed. Nutr Cancer 1997;29:222-7.
20. Pasternak CA. An Introduction to Human Biochemistry. Edn 1, Oxford University Press, New York 1979, 271.
21. Patrick L. Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. Altern Med Rev 2006;11(1):2-22.
22. Pokras MA, Kneeland MR. Understanding lead uptake and effects across species: A conservation medicine based approach. Proceeding of 2008 Lead Ammunition Conference, Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. Idaho, USA 2009, 1-16.
23. Pradyumna RT, Okamoto T, Akita N, Hayashi T, Kato-Yasuda N, Suzuki K. Amla (Emblica officinalis Gaertn.) extract inhibits lipopolysaccharide induced procoagulant and pro-inflammatory factors in cultured vascular endothelial cells. Br J Nutr 2013;7:1-6.
24. Prasad K. Flaxseed: a source of hypocholesterolemic and antiatherogenic agents. Drug News Perspect 2004;13(2):99-104.
25. Qureshi N, Sharma R. Lead toxicity and infertility in female Swiss mice: A review. J Chem Biol Phys Sci 2012;2(4):1849-1861.
26. Ramanathan K, Anasuyadevi M, Shila S, Panneerselvam C. Ascorbic acid and alfa tocopherol as potent modulators of apoptosis on arsenic-induced toxicity in rats. Toxicol Lett 2005;156:297-306.
27. Rhee Y, Brunt A. Flaxseeds supplementation improved insulin resistance in obese glucose intolerant people: a randomized crossover design. Nutr J 2011;10:44.
28. Sekine S, Sasanuki S, Murana Y, Aoyama T, Takeuchi H. Alpha- linolenic acid rich flaxseed oil ingestion increases plasma adiponectin level in rats. Int J Vitam Nutr Res 2008;78:223-229.
29. Serbecic N, Beutelspacher SC. Anti-oxidant vitamins prevent lipid- peroxidation and apoptosis in corneal endothelial cells. Cell Tissue Res 2005;320:465-475.
30. Sharma V, Kansal L, Sharma A, Lodi S, Sharma SH. Ameliorating Effect of *Coriandrum sativum* Extracts on Hematological and Immunological Variables in an Animal Model of Lead Intoxication**.** J Pharm Allied Health Sci 2011;1(1):16-29.
31. Snedecor WG, Cochran GW. Statistical Methods. Edn 6, Oxford and IBH Publishing Company, New Delhi 1994, 258-268.
32. Sujatha K, Srilatha Ch, Anjaneyulu Y, Chandra Sekhara Rao TS, Sreeni Vasulu D, Amravathi P. Hematobiochemical changes of lead Poisoning and amelioration with Ocimum sanctum in wistar albino rats. Vet World 2011;4(6):260-263.
33. Taupeau C, Poupon J, Nome F, Lefevre B. Lead accumulation in the mouse ovary after treatment-induced follicular atresia. Reprod Toxicol 2001;15:385-391.
34. Tham DM, Gardner CD, Haskell WL. Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. J Clin Endocrinol Metab 1998;83:2223-35.
35. Troina AA, Figueriedo MS, Moura EG, Boaventura GT, Soares LL, Cardozo LF. Maternal flaxseed diet during lactation alters milk composition and programs the offspring body composition, lipid profile and sexual function. Food Chem Toxicol 2010;48:697-703.
36. Wang C, Liang J, Zhang C, Bi Y, Shi S. Effect of Ascorbic Acid and Thiamine Supplementation at Different Concentrations on Lead Toxicity in Liver. Ann Occup Hyg 2007;51(6):563-569.
37. Waseem N, Rehman S. The Efficiency of Garlic Extract in Prevention of Lead Acetate Toxicity on Fallopian Tube-A Hormonal Study. Biol Med 2015;**7**:227.
38. Waseem N, Hamid S, Butt SA. Effect of lead acetate on follicular count of mice ovary and the protective role of garlic extract. Pak Armed Forces Med J 2014;64(1):61-65.
39. Wiart C. Note on the relevance of *Emblica officinalis* Gaertn. For the treatment and prevention of cancer. Eur J Cancer Prev 2013;22(2):198.
40. Zanwar AA, Hedge MV, Bodhankar SL. Cardioprotective activity of flax lignan concentrate extracted from seeds of *Linum usitatissiumum* in isoprenalin induced myocardial necrosis in rats. Interdiscip Toxicol 2011;4:90-97.
41. Zhang Y, Abe T, Uang C, Kouno I. Phyllanemblinins A-F, New ellagitannins from *Phyllanthus emblica*. J Nat Prod 2001;64:1527-153.
42. Zhang Z, Li D, Wang L, Ozkan N, Chen XD, Mao Z, Optimization of ethanol-water extraction of lignans from flaxseed. Sep Purif Technol 2007;57:17-24.