Explor Anim Med Res,
Vol.12, Issue - 2, 2022, p. 264-272
DOI : 10.52635/eamr/12.2.264-272

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

CONCURRENT EFFECT OF *LINUM USITATISSIMUM* AND *EMBLICA OFFICINALIS* ON LEAD INDUCED OXIDATIVE STRESS AND HISTOMORPHOLOGICAL CHANGES IN UTERUS OF FEMALE WISTAR RATS

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ABSTRACT: Lead (Pb) is one of the oldest environmental contaminants and is well known to cause deleterious systemic effects on man and domestic animals including the reproductive system. The present study aimed to investigate the impact of the combined administration of aqueous methanolic extract of *Linum usitatissimum* (Flaxseed) and aqueous extract of *Emblica Officinalis* (Amla) on lead-induced oxidative stress and pathological changes in the uterus of wistar rats. A total of 108 female adult wistar albino rats were randomly assigned to 6 groups with 18 rats in each group. Group I served as vehicle control and they received distilled water, group II was fed with lead acetate @ 60 mg/ kg b.wt., group III provided with *Emblica Officinalis* @ 100 mg/ rat/ day, group IV was given *Linum usitatissimum* @ 300 mg/ kg b.wt, group V had 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. for 45 days. In this study, thiobarbituric acid reactive substances (TBARS) increased and decreased levels of antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels in the uterus were noticed in the lead-treated group. Histopathology of the uterus showed severe degenerative changes and immunohistochemistry (IHC) with BAX markers in the uterus revealed increased expression with BAX markers in lead treated rats. The levels of all the above parameters were significantly improved in the ameliorated group (Groups V and VI). The observations made in the study indicated that treatment of amla and flaxseed in rats concurrently with the lead was shown to have an ameliorating effect and amla was found to be relatively better than that of flaxseed in amelioration of different pathological manifestations against lead-induced toxicity.

Key words: Amla, BAX, CAT, Flaxseed, GPx, Immunohistochemistry, Lead, SOD, TBARS.

INTRODUCTION

Lead is one of mankind's oldest environmental and occupational toxins having detrimental effects on the growth, health, reproductive performance, and life span of all living beings. Lead intoxication is one of the most frequently encountered poisoning in veterinary medicine worldwide, recorded in all domestic and several zoo species (Patrick 2006, Gupta 2007). Lead exhibits toxic effects through inhibition of enzymatic function, generation of oxidative stress, interference with the action of essential cations, modification of cell signaling, and disruption of the integrity of cellular membranes or organelles (Gurer and Ercal 2000, Nakade *et al.* 2015). From the viewpoint of reproduction, lead is known to cause a number of adverse consequences in both humans and animals. Several studies suggested that low doses of lead affect sexual development and reproductive dysfunctions in men, women, and small mammals either directly or indirectly (Waseem and Rehman 2015, Nakade *et al.* 2015).

Over the years research has thrown light on the use of medicinal plants as they have been identified as sources of various phytochemicals with a wide variety of medicinal properties. The use of *Emblica officinalis* Gaertn., Family: Phyllanthaceae (emblica or amla) has been reported in Indian traditional medicine since ancient times (Senthil *et al.* 2008). Emblica extract is proven to be a potent antioxidant, anti-inflammatory, and chemoprotective agent (Wiart 2013, Golechha *et al.* 2012). Tannaoids are the main active principles present in amla having vitamin C-like properties that produced anti-inflammatory, a potent antioxidant, and anti-mutagenic effects (Ghosal *et al.* 1996). *Linum usitatissium* L., Family: Linaceae (flaxseeds), commonly known as linseed is the richest source of phytoestrogenic and anticancer compounds

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referred to as lignans (Milder *et al.* 2005). Additionally, flaxseed has antioxidant, cardioprotective, chemo-protective, hepatoprotective, and anti-inflammatory properties (Zanwar *et al.* 2011, Rhee and Brunt 2011, Troina *et al.* 2010).

In the past few decades, many researchers have explored that the supplementation of herbal agents like garlic, curcumin, and vitamin-c not only confer protection against heavy metal toxicity but also can be of therapeutic importance against toxicity (Waseem and Rehman 2015, Abdou and Hassan 2014). As free radicals are generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy (Gurer and Ercal 2000, Ghosal *et al.* 1996). Herbal agents have minimal to no side effects compared to synthetic chelating agents and possess various health benefits. Hence, keeping in view the beneficial effect of herbal extract in this present study amla and flaxseed were taken into consideration for evaluating the possible protective nature against lead toxicity.

Lead toxicity is a serious worldwide problem and is considered to be the most common occurrence in both animals and human beings. With the development of industrialization and human activities, the discharge of waste and wastewater containing heavy metals into the environment has increased. Accidental ingestion or licking of lead objects such as batteries, gunshots, lead-based paints, contaminated pastures and vegetation, agricultural use of fertilizers, lead pesticide sprays, and inhalation of fumes from burning storage batteries are main sources of poisoning in case of animals. Lead absorption occurs mainly by inhalation and ingestion and very little amount through the skin. Lead is thus absorbed mainly stored in soft tissues such as the liver, kidney, CNS, blood, and chronic exposure results in the release of lead into the skeletal pool (Patrick 2006). Animal models are widely used to characterize the toxicity of different toxicants and to study the ameliorating effects of many herbs. The present study mainly focused on the reproductive toxicity aspect of lead and its possible amelioration by the herbal extract.

MATERIALS AND METHODS

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

Female Wistar albino rats (*Rattus norvegicus,* outbred strain) with body weights of around 180 to 200 gms (Sri Venkateswara Enterprises, Bangalore) were used for the present experiment. After one week of the acclimatization period, the rats were grouped randomly and housed in standard polypropylene rat cages (three rats/cage). They were maintained at 25° C ± 5°C and a 12:12 hour interval light and dark cycle during the 45 days of the experimental period by maintaining standard laboratory hygienic conditions with *ad libitum* supply of feed (Nutrimix STD-1020 rat pelleted feed) and water (Sherwin 2004). The permission of the institutional animal ethical committee (IAEC) was obtained before the commencement of the experiment (Reference number- 281/go/ReBi/S/2000/ CPCSEA/CVSC/TPTY/018/VPP/2016-17). The Lead acetate (product code No.27645, 97% purity) was obtained from Qualigens Fine Chemicals and an aqueous extract of Amla (*Emblica officinalis*) (product code C/ SVU/EMOF-01) was supplied by Chemiloids, Vijayawada, India.

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

Seeds of *Linum usitatissimum* (flax seeds) were obtained from a local herbal shop. After drying in shade, seeds were ground into powder form, which was then used for the preparation of aqueous methanolic extract (Zhang *et al.* 2007). Around 800g of flaxseed were ground into powder form and defatted by blending with hexane (Thermo Fisher) (1:6 w/v, 12 h) at room temperature (25 °C). The defatted flaxseed powder was air-dried for around 12 hours. 200 grams of this defatted powder was again blended with a 1.2 litre complex solution of methanol and water (7:3 v/v) for 24 hours at ambient temperature (25 °C). The extract was filtered into a flask, and then the filtered product was concentrated at 50 °C using a Rotary evaporator (Buch, Germany) @ 90 rpm and light yellow color syrup of flaxseed lignans extract was obtained. Further, the yellow semisolid extract of flaxseed was air-dried to obtain a fine powder form (Zhang *et al.* 2007).

Experimental design

A total of 108 female adult wistar albino rats of 2 months age were randomly assigned to 6 groups with 18 rats in each group. The groups were treated as follows:

Group I: Control, without any medication.

Group II: Lead acetate @ 60 mg/ kg b.wt. ($1/10^{th}$ of LD50 in wistar rats).

Group III: *Emblica officinalis* @ 100 mg/ rat/ day.

Group IV: *Linum usitatissimum* @ 300 mg/ kg b.wt.

Group V: Lead acetate @ 60 mg/ kg b.wt + *Emblica Officinalis* @ 100 mg/ rat/ day.

Group VI: Lead acetate @ 60 mg/ kg b.wt + *Linum usitatissimum* @ 300 mg/ kg b.wt.

Rats were clinically monitored throughout the

experimental period of 45 days. 6 rats from each group were sacrificed (chloroform anesthesia) at a 2-week interval.

Histopathology

Following a detailed post-mortem examination of the sacrificed rats, the gross changes were documented. Uterus was collected and preserved in 10% neutral buffered formalin (NBF) for histopathological and immunohistochemical (IHC) studies (Sujatha *et al.* 2011). Formalin-fixed tissues were processed and stained following the standard method. Haematoxylin and eosin stained (H&E) tissue sections were examined under a microscope and lesions were recorded (Bancroft and Gamble 2008).

Lipid peroxidation (TBARS) assay and antioxidant profile

For estimation of antioxidant enzymes fresh uterus was collected and stored at -40° C until use. Tissue pieces of the uterus were minced in a container and homogenized in 0.05 M ice-cold phosphate buffer (pH 7.4) and made 10% homogenate by using a Virtis homogenizer. 0.2 ml of the 10% homogenate was utilized for lipid peroxidation assay (Yagi 1976). The residual part of homogenate was then mixed with 10% trichloroacetic acid in a 1:1 ratio, then centrifuged @ 5000 g at 4°C for 10 min and the supernatant was collected and was used for valuation of reduced glutathione (Moron *et al.* 1979). The residual portion of the homogenate was centrifuged (Eppendorf) for 60 min. at 15,000 g at 4°C and the supernatant attained was used for the valuation of superoxide dismutase (Marklund and Marklund 1974), catalase (Caliborne 1985), and glutathione peroxidase (Rotruck *et al.* 1973).

Immunohistochemistry

To assess the apoptosis in the uterus immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections using a BAX marker (BCL2-Associated X protein). The primary and secondary antibodies were obtained from Biogenex Company. Tissue sections of 5µ thickness were taken into slides pre-coated with APES (Aminopropyltriethoxysilane, Sigma-Aldrich). Slides were dewaxed and rehydrated in xylene and a graded series of alcohol. Antigen unmasking was done by treatment with Proteinase K (Bangalore Genei) and endogenous peroxidase was quenched by keeping the tissue sections in a peroxidase block solution for 30 min. The primary antibody was applied overnight at 4°C. HRPO conjugated secondary antibody (BAX) was applied for 30 min at room temperature and washed in PBS. DAB substrate was added in tissue sections with continuous monitoring under a microscope followed by washing in PBS to stop the reaction, counter-stained with Harris haematoxylin and finally mounted in permanent mounting media (Jin *et al.* 2008).

Statistical analysis

The results were analyzed by using SPSS statistical software (statistical package for social sciences) (Paul *et al.* 2022) through one-way ANOVA (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Clinical sign and behavioural change

Clinical signs like reduced feed intake decreased growth rate, anxiety, ruffled hair coat, and nervous symptoms like hyper tetchiness and scratching of the face were noted in lead acetate-treated rats (Group-II) from the 4th week onwards. Similar clinical signs were observed by previous authors (Paul *et al.* 2021, Aline *et al.* 2007) and this might be due to the penetration of lead into blood brain-barrier, inhibition of heme synthesis, and transport protein (transferring) synthesis (Alwaleedi 2016, Wang *et al.* 2013). Group III (Emblica treated) and group IV (Flaxseed treated) were normal without any clinical signs and no mortality was recorded in any of the treated groups during the entire experimental period.

Lipid peroxidation – Thiobarbituric acid reactive substances (TBARS)

The current study revealed a significant ($p< 0.05$) increase in mean uterus TBARS levels in lead-treated rats (Group II) as compared to the control rats (Group I). The mean uterus TBARS values from group I to VI were 559.83, 1431.8, 568.4, 567.1, 591.5, and 601.4 (nM of MDA/ g of tissue) respectively, and presented in Table 1. Similar observations were described by earlier authors (Paul *et al.* 2022, Paul *et al.* 2021). The possible explanation is associated with the role of glutathione (GSH) (El-Nekeety *et al.* 2009). Lead (Pb) binds with the thiol group present in GSH, and thus plays an important role in the active excretion of lead through bile (Upasani *et al.* 2001). Lead induces direct depletion of antioxidant enzymes [Superoxide dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase (CAT)] which are essential in maintaining GSH homeostasis in tissues. So depletion of these antioxidant enzymes decreases GSH levels in tissues which ultimately lead to oxidative stress and a subsequent increase in lipid peroxidation (Ponce-Canchihuaman *et al.* 2010).

Emblica ameliorated group (Group V) showed a

significant decrease in lipid peroxidation activity compared to group II and this might be due to antioxidant properties and reduction of lipid peroxidation activity due to the tannoid rich fraction of emblica including emblicanin-A and B (Sultana *et al.* 2005, Prasad 2000). Flaxseed ameliorated rats (Group VI) showed significant ($p<0.05$) improvement in lipid peroxidation activity when compared to lead-treated groups and this is probably due to the lipid-lowering effect and reduction of tissue lipid peroxide (MDA) properties of flaxseed (Zanwar *et al.* 2011, Lee and Prasad 2003).

Antioxidant profile

To evaluate the lead-induced oxidative stress, antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were analyzed in the uterus, and a statistically significant ($p< 0.05$) decrease in mean antioxidant enzymes values were noticed in the lead acetate treated rats (Group II) compared to the control rats (Group I). The mean uterus catalase activity from group I to VI were 0.31, 0.12, 0.32, 0.31, 0.28, and 0.26 (nM of H2O2 decomposed/ min/ mg of protein) respectively, and are shown in Table 2. The mean uterus SOD activity in Groups I to VI were 15.2, 7.3, 15.23, 15.13, 14.66, and 14.16 (U/ min/ mg of protein) respectively, and presented in Table 3. The mean uterus GPx activity from group I to VI were 23.27, 12.2, 23.47, 23.02, 18.99, and 18.44 (ì of glutathione utilized/ min/ mg protein) respectively, and presented in Table 4. These findings were in agreement with previous researchers (Paul *et al.* 2022, Wang *et al.* 2006). The decreased levels of antioxidant enzymes might be an outcome of lead-induced excessive production of reactive oxygen species (ROS), reduction in antioxidant cell defense system by depleting GSH, inhibition of sulfhydryl-dependent enzymes, or by interfering with some important metals like copper required for proper activities of antioxidant enzyme (Gupta 2007, Aline *et al.* 2007).

Experimental findings revealed that in group V and group VI rats, there was a statistically significant increase in mean uterus antioxidant enzymes level compared to group II. Significant increase in SOD, GPx, and CAT levels in Group V rats might be due to the antioxidant property of the tannoid rich fraction of emblica including emblicanin A and B (Tasanarong *et al.* 2014, Alghazal *et al.* 2008). Significant increase in SOD, GPx, and CAT levels in Group VI rats, probably owing to the presence of Omega-3 fatty acid and secoisolariciresinol diglucoside (SDG) in flaxseed which exerts antioxidant properties, helps in counter the lead-induced oxidative stress and subsequently increases the level of antioxidant enzymes (Zanwar *et al.* 2011, Lee and Prasad 2003). Whereas, there was no significant alteration in lipid peroxidation (TBARS) and antioxidant enzymes level (CAT, SOD, and GPx) in emblica (Group III) and flaxseed (Group IV) treated rats compared to control groups (Group-I).

Gross and histopathological appearance

Conspicuous gross lesions were noticed in the uterus. From the 4th week onwards slight reduction in the size of the uterus was observed in lead acetate-treated (Group II) rats and this reduction was more prominent at the end

Table 1. Mean uterus TBARS values (nM of MDA /g of tissue) in rats of different experimental groups.

Weeks	Group I	Group II	Group III	Group IV	Group V	Group VI
2	570.6	1385.2	570.2	568.3	590.2	598.2
4	550.3	1429.5	563.8	562.3	588.5	608.7
6	558.6	1480.7	571.2	570.7	595.8	597.3
Mean ±S.E	559.83 ±5.89^{c}	1431.8 ±47.79^{a}	568.4 ±2.3^{bc}	567.1 ±2.49^{bc}	591.50 ±2.20^{bc}	601.4 ±3.65^{b}

*Mean values with different superscripts differ significantly ($p < 0.05$), ANOVA, S.E- Standard error.

Table 2. Mean uterus catalase activity (nM of H_2O_2 decomposed/ min/ mg of protein) in rats of different experimental groups.

Weeks	Group I	Group II	Group III	Group IV	Group V	Group VI
2	0.30	0.16	0.31	0.30	0.27	0.24
4	0.32	0.12	0.32	0.31	0.28	0.27
6	0.33	0.08	0.34	0.32	0.30	0.29
Mean ±S.E	0.31 ±0.008^{a}	0.12 ±0.02^{c}	0.32 ±0.008^{a}	0.31 ±0.005^{a}	0.28 ±0.008^{ab}	0.26 ±0.01^{b}

*Mean values with different superscripts differ significantly ($p < 0.05$), ANOVA, S.E- Standard error.

Table 3. Mean uterus SOD activity (U/ min/ mg of protein) in rats of different experimental groups.

Weeks	Group I	Group II	Group III	Group IV	Group V	Group VI
2	14.9	7.8	15	15	14.4	13.9
4	15.1	7.2	15.3	15.1	14.7	14.2
6	15.6	6.9	15.4	15.3	14.9	14.4
Mean ±S.E	15.2 ±0.2 [a]	7.3 ±0.26 [c]	15.23 ±0.12 [a]	15.13 ±0.08 [a]	14.66 ±0.14 [ab]	14.16 ±0.14 [b]

*Mean values with different superscripts differ significantly ($p < 0.05$), ANOVA, S.E- Standard error.

Table 4. Mean uterus glutathione peroxidase activity (µ of glutathione utilized/ min/ mg of protein) in rats of different experimental groups.

Weeks	Group I	Group II	Group III	Group IV	Group V	Group VI
2	20.82	16.53	21.37	20.74	18.59	18.15
4	23.48	13.21	23.28	22.68	18.95	18.39
6	25.52	7.03	25.78	25.65	19.45	18.78
Mean ±S.E	23.27 ±1.36[ab]	12.2 ±2.7 [c]	23.47 ±1.2 [a]	23.02 ±1.4 [ab]	18.99 ±0.24 [ab]	18.44±0.18[b]

*Mean values with different superscripts differ significantly ($p < 0.05$), ANOVA, S.E- Standard error.

of 6th week of the experiment (Fig. 1). Whereas in the uterus of ameliorated rats (Group V and VI) showed no apparent changes during the present study period.

The present study revealed various degrees of histological changes that accompanied the increased level of lipid peroxidation and decrease level of antioxidant enzymes in the uterus of lead-treated rats (Group II) as compared with those of the control group (Group I). These changes were duration dependent; the longer the duration the more damaging effects.

Histopathological investigation of lead-treated rats (Group II) revealed degenerative changes in endometrial lining epithelium, mild periglandular fibroblast proliferation in the endometrium, and mild infiltration of eosinophil in between muscle bundles of the myometrium by the end of 2nd week. Additionally, disruption of endometrial glandular structure and perivascular infiltration of eosinophil in the endometrium, and mild distortion of smooth muscle fibers in the myometrium were recorded by the end of 4th week of the experiment. Later stages (6th week) of the experiment, the degrees of changes were more severe like completely degenerated and distorted endometrial glands with periglandular fibrous tissue proliferation (Fig. 2), desquamation of lining columnar epithelium, variation in size, shape, and reduction in several endometrial glands along with cystic dilatation (Fig. 5,6) and narrowing of the uterine lumen (Fig. 3). Whereas thinning of the myometrium with severe eosinophilia in between muscle bundles and disruptions of smooth muscle bundles (Fig. 4) were more evident in the myometrium. Similar observations were made by earlier researchers (Nakade *et al.* 2015). The changes in uterine histology are probably due to lead-induced alteration in uterine glandular secretions like enzymes, growth factors, hormones, cytokines, lymphokines, and different transport proteins which hampers the normal in-utero physiological functions and subsequent pathophysiological changes in uterine tissue (Patrick 2006, Spencer and Bazer 2004). Lead-induced alteration in the dynamics of uterine membrane receptors and ion channels is another probable reason for histological changes in the uterus (Nakade *et al.* 2015).

In group V (lead + amla) and group VI (lead + flaxseed) rats, the lesions as described in group II were noticed up to 2nd week of the experiment. Later the changes were gradually decreased in its intensity with a restored number of endometrial glands with very mild degenerative changes in glandular epithelium. Myometrium revealed, an almost regularly arranged smooth muscle layer and normal thickness with mild infiltration of eosinophil. The uterus almost regained its normal appearance with a restored number of endometrial glands and normal size of uterine lumen (Fig. 7, 8, 9, 10) by the end of the experimental period (6th week). The improvement in histopathological alteration of group V rats might be due to the rich fraction of vitamin C present in emblica which would effectively protect endometrial tissue from heavy metal-induced damage (Tasanarong *et*

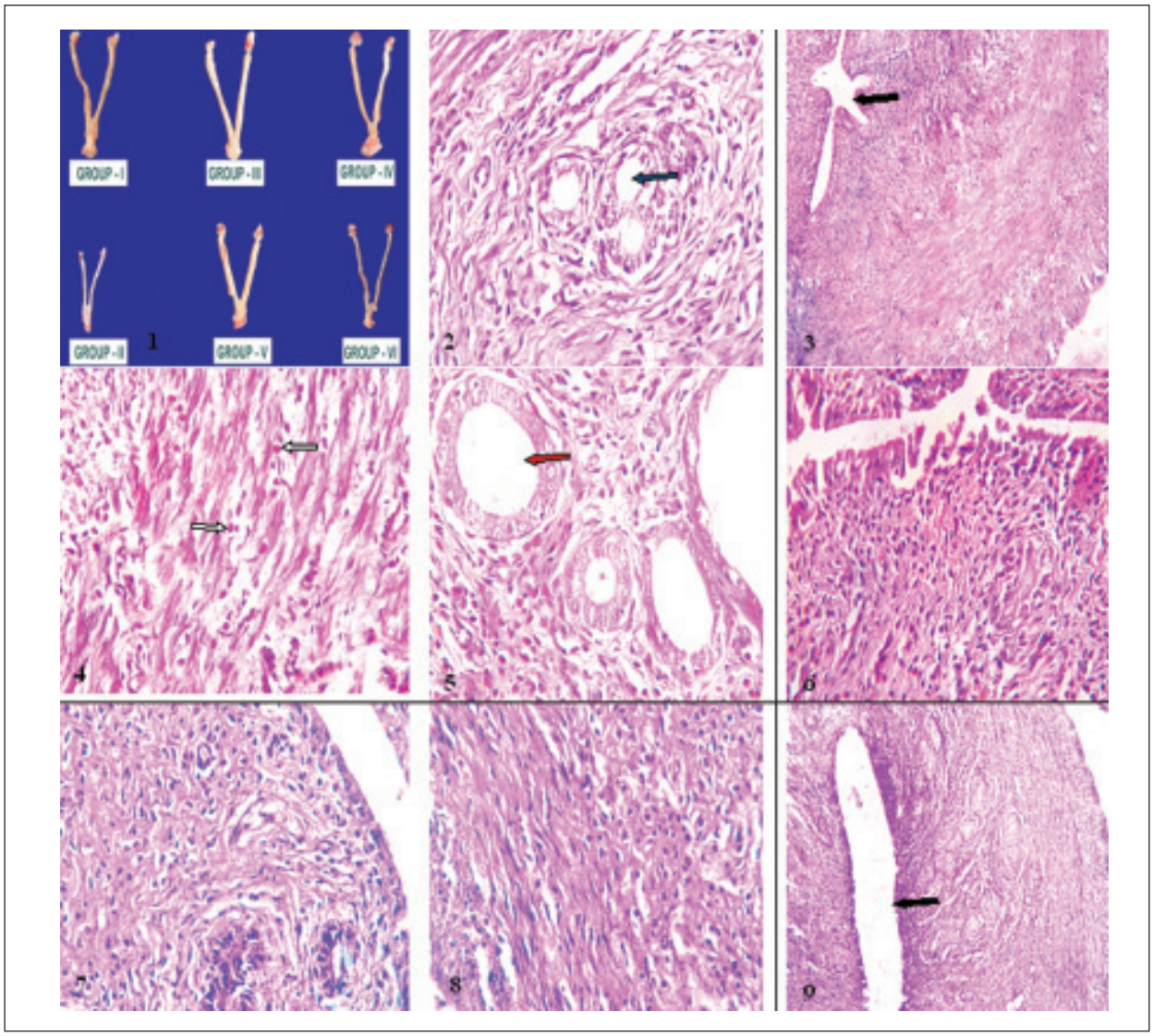

Fig. 1-9. (left to right): Fig. 1. Decreased size of uterus in lead treated rats (Group-II) whereas normal uterus in other treatment group. Fig. 2. Group II: Uterus: Degenerated endometrial glands (blue arrow) and proliferation of fibrous connective tissue around the endometrial glands. H & E x 400. Fig. 3. Group II: Uterus: Reduced size of uterine lumen (black arrow). H & E x 40. Fig. 4. Group II: Uterus: Degenerative changes of smooth muscle bundles in myometrium and infiltration of eosinophil (white arrow). H & E x 400. Fig. 5. Group II: Uterus: Section showing cystic dilatation of endometrial glands (red arrow) with degenerated glandular epithelium. H & E x 400. Fig. 6. Group II: Uterus: Section showing desquamated lining columnar epithelium. H & E x 400. Fig. 7. Group V: Uterus: Note normal appearance of endometrium and the lining epithelium. H & E x100. Fig. 8. Group V: Uterus: Note normal arrangement of smooth muscle bundles with normal thickness of myometrium. H & E x 400. Fig. 9. Group V: Uterus: Note restoration of normal number of endometrial glands (black arrow). H & E x 100.

al. 2014, Alghazal *et al.* 2008, Guney 2007). Likewise, flaxseed ameliorated groups (Group VI), uterus regained almost near normal structure, and this is probably due to the presence of lignan mainly secoisolariciresinol diglucoside (SDG) in flaxseed which has antioxidant and phytoestrogen properties (Adolphe *et al.* 2010, Sekine *et al.* 2008). There was no significant gross and microscopic alteration was observed in the uterus of emblica (Group III) and flaxseed (Group IV) treated rats compared to control group rats.

Immunohistochemistry

Immunohistochemistry was done for the assessment of apoptotic changes in the uterus by using monoclonal antibodies against apoptosis marker BAX antigens, as this antigen is well expressed in cells undergoing apoptotic changes (Sujatha *et al.* 2011, Jin *et al.* 2008). Intense brown color development in tissue sections of the uterus specifies the presence of BAX antigen. The uterus of control Group rats (I) showed minimal to mild

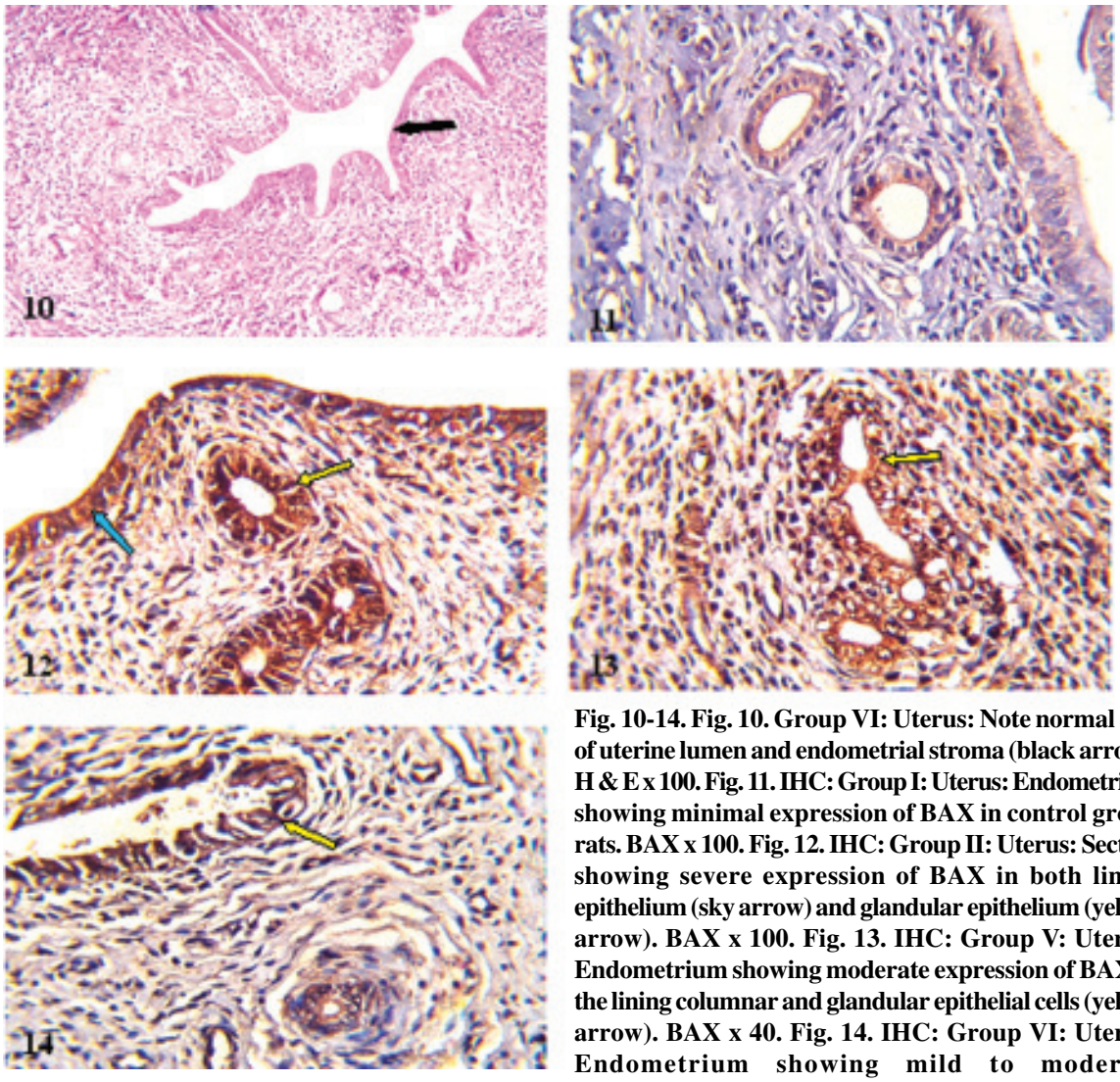

Fig. 10-14. Fig. 10. Group VI: Uterus: Note normal size of uterine lumen and endometrial stroma (black arrow). H & E x 100. Fig. 11. IHC: Group I: Uterus: Endometrium showing minimal expression of BAX in control group rats. BAX x 100. Fig. 12. IHC: Group II: Uterus: Section showing severe expression of BAX in both lining epithelium (sky arrow) and glandular epithelium (yellow arrow). BAX x 100. Fig. 13. IHC: Group V: Uterus: Endometrium showing moderate expression of BAX in the lining columnar and glandular epithelial cells (yellow arrow). BAX x 40. Fig. 14. IHC: Group VI: Uterus: Endometrium showing mild to moderate immunoreactivity with BAX in the lining epithelium and glandular epithelium (yellow arrow). BAX x 400.

immunoreactivity (Fig. 11) whereas increased expression of BAX antigen in the glandular lining epithelium of the endometrial gland, lining columnar epithelium of the uterus of lead acetate treated rats (Group II) (Fig. 12) was seen in lead treated rats (Group II). Similar observations were made by previous authors (Paul *et al.* 2021, Jin *et al.* 2008). This is probably due to lead-induced alteration in the cell respiration, inhibition of energy production and oxidative damage resulting increase in the expression of apoptosis relate protein (Jin *et al.* 2008).

In emblica ameliorated rats (Group V) decreased expression of BAX antigen was observed in the uterus (Fig. 13) and this might be associated with the protective role of vitamin C present in emblica that blocks the apoptotic pathway (Serbecic ang Beutelspacher 2005, Ramanathan *et al.* 2005). Decreased expression of BAX antigen was observed in the uterus of flaxseed ameliorated rats (Group VI) (Fig. 14). This is due to the presence of Omega-3 fatty acid and secoisolariciresinol diglucoside (SDG), which exerts antioxidant properties (Adolphe *et al.* 2010, Sekine *et al.* 2008) and protect cells from oxidative stress-induced damage.

CONCLUSION

The observations made in the study indicate that the administration of lead (Pb) resulted in various pathological changes in the reproductive organ; the uterus and these changes were aggravated by an increase in lipid peroxidation and a decrease in antioxidant enzyme levels. Supplementation of emblica and flaxseed concurrently with the lead was shown to have an ameliorating effect on different pathological manifestations, decreased lipid

peroxidation rate, and improved the status of antioxidant enzymes level in the flaxseed and amla ameliorated rats group. The ameliorating effect of emblica was found to be relatively better than that of flaxseed in most of the parameters. The results of this investigation infer that the continuous use of emblica and flaxseed in day-to-day life might have therapeutic importance as supportive therapy in combination with chelation therapy to reduce the harmful and toxic effects of heavy metals like lead.

ACKNOWLEDGEMENT

The authors are thankful to the Dean, of the College of Veterinary Science, Tirupati, for providing all the facilities during the research period.

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Cite this article as: Paul A, Sujatha K (2022) Concurrent effect of *Linum usitatissimum* and *Emblica officinalis* on lead induced oxidative stress and histomorphological changes in uterus of female Wistar rats. Explor Anim Med Res 12(2): 264-272. DOI: 10.52635/eamr/12.2.264-272.