ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# Effect of Lanthanum on Pro-Angiogenic Growth Factors in Chick Embryos

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## *Manuscript Info*

***Manuscript History:***

Received: 12 April 2014
Final Accepted: 23 May 2014
Published Online: June 2014

***Key words:***
Lanthanum, TGFβ2, GDNF, Immunohistochemical, Chick embryos

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## *Abstract*

Lanthanum (**La**) compounds are widely used in optics, electronics and therapeutics. The present study was undertaken to establish the experimental animal model the chick embryos for **La** toxicity and its effect on pro-angiogenic growth factors (TGFβ2 and GDNF) as members of transforming growth factor superfamily. In fertilized eggs, **La** was injected through the air chamber once time and the eggs were incubated prior to control for 10 days. At $7^{th}$ and $10^{th}$ day for control and at the $10^{th}$ day for **La**-exposed, embryos were dissected out to follow the morphological abnormalities and the differentiation of ciliary body, spinal cord, lung, liver and kidney. Results indicated that **La** exerts a dose-dependent toxicity, retarding the differentiation of ciliary body, decrease the extracellular matrix and down regulate the expression of the growth factors under investigation. The study concluded that beside the therapeutic role of **La** in hyperphosphatemia, the studied growth factors by their angiogenic activity may contribute to the advent of a new generation of metal agents that directly down regulate their expression with consequent inhibition of tumor metastasis through **La** therapeutic uses.



## INTRODUCTION

Rare earth (RE) metals include 17 elements that are categorized into two groups. One is called light which is represented by cerium including lanthanum and the other is heavy which is represented by yttrium. Recently, RE metals has become one of the common xenobiotics in our surroundings as it is widely used in industry, stockbreeding, medicine, agriculture and they concentrated by food chain. Studies on RE metals toxicology have lasted for a long time. However, deeper exploration of its mechanism is highly needed. It is well known that the biological effect spectrum of RE metals is wide and the dose-response relationship is complicated. RE elements could induce damage of liver (Nakamura et al., 1997), chromosome of blood lymphocytes (Xu et al. 2000), depress learning and memory (Li et al., 2000), increase or suppress cell-mediated immunity of the spleen (Liu et al., 2000) and inhibit gap junctional intercellular communication (Guo et al., 2000). RE metal lanthanum is a silvery white metallic element that belongs to group 3 of the periodic table and it is the first element of the lanthanide series. Lanthanum has been widely used in pharmacological, agriculture and in electronic industries. Calcium channel blocker, dental caries prevention, trace fertilizer, optical and semiconductor application are the most uses of lanthanum in various fields (Das et al., 1988; Guo, et al., 1990). The element is very poorly absorbed after oral administration and its elimination is very slow. Great use of lanthanum in industrial, agricultural and medication increase the chances of accidental exposure at both acute and chronic levels. In this context, long term intake of low-dose lanthanides was found to induce a significant negative impact on the signal conduction velocity in the brain of adults and decrease intelligence of children's whom lived in lanthanide area (Zhu et al., 1997; Briner et al., 2000; Fan et al., 2004). In early studies, the developmental toxicity of lanthanum in mice and rats was investigated.

Reduction in the number of successful pregnancies, average litter size, alteration of visual response, defects in walking behavior and impairment of the learning and abilities of rats as a result of prenatal exposure of lanthanum were recorded (Abramczuk, 1985; Feng et al., 2006). In MCF-7 cell line, lanthanum chloride repressed cell proliferation at high concentrations and was found to play a clue for inhibiting the growth and invasion of tumor (Li et al., 2009).

From the other hand, the development of new blood vessels out of existing ones (angiogenesis) is a fundamental requirement for new organ development and for differentiation during embryogenesis, wound healing and reproductive physiology of adults (Folkman and Klagsbrun, 1987; Folkman, 1990). Angiogenesis is also takes place in some pathologic conditions such as rheumatoid arthritis, proliferative retinopathies as well as tumor growth and metastasis (Rosen, 2002). So, the present investigation aims to evaluate, rather than the toxicological effects, the effect of lanthanum on two of the transforming growth factor super family members, the Transforming Growth Factor beta2 (TGF β2) and the Glial Cell Line-Derived Neurotropic Factor (GDNF) as the most growth factors involved in angiogenesis for proper development and in pathologic conditions (Kloth and Sauter-Crazzolara 2000). For these tasks, chick embryos were used to avoid the materno-placental effects in mammalian models as the embryo develops independently of the hen and allows experimentation without maternal influences.

## MATERIALS AND METHODS

Lanthanum

Lanthanum was obtained commercially in the form of chloride salt ($LaCl_3$. $7H_2O$). In NaCl saline solution (0.9g/100ml), stock solution of lanthanum in bi-distilled water was prepared for use.

Fertile chicken eggs

Fertile eggs of Egyptian Fayoumi (Gallus gallus domisticus) were obtained from Chicken Culture Station of species preservation at Sohag, Egypt.

Experimental design

For experimental adjustment, a preceding toxicity test was conducted in which chicken's fertilized eggs were exposed to lanthanum (50, 100 and 150 µg/egg in 0.2ml solution) by injection through the air chamber in addition to the control group 30 egg each of three replicates. Injection of lanthanum was carried by a single dose only one time before the onset of incubation during the experimental period up to 10 days of incubation at 38˚C and 50 – 60 % humidity. The percentage of resorption was recorded and embryos of various groups were photographed for morphological evaluation.

Based on the observed embryonic death of the preceding experiment, the eggs were classified into two groups: control and 50µg/egg lanthanum-injected groups. The control group received saline (0.2 ml/egg) while the treated groups received lanthanum solution corresponding to the concentrations used in the same volume (0.2 ml/egg) through injection via the air chamber. Embryos of control at the $7^{th}$ and $10^{th}$ days and those of lanthanum-exposed group at the $10^{th}$ day of incubation were examined. Selected embryos after insurance of their viability at the above indicated days for both the control and lanthanum-exposed were fixed in Carnoy's fixative and processed for section cutting. Sections of 7µm thick were stained with hematoxylin and eosin for general histology, periodic acid Schiff's reaction for polysaccharide contents, alcian blue (pH 2.6) for acid mucopolysaccharides and acridine orange to investigate the nuclear viability with fluorescent microscope (Drury and Wallington, 1976). Morphogenesis of ciliary body of the developing eye, spinal cord, lung, liver and kidney was examined in serial sections of control compared to lanthanum-exposed group.

For immune-histochemical investigation, positive slides mounted sections were deparaffiniezed and retrieved for antigen reantigenicity using 10 mM citrate buffer (pH6) at 100 ˚C for an hour (Buchlowalow and Bocker, 2010). Sections were treated for 10 minutes with hydrogen peroxide block (0.3% hydrogen peroxide solution with less than 0.1% sodium azide) and then with protein block (phosphate buffer solution, pH 7.6, with 0.5% BSA, 0.5% casein and less than 0.1% sodium azide) for 10 minutes to block nonspecific background staining. Primary antibodies, rabbit anti-human polyclonal TGFβ2 (1:100 dilution) in phosphate buffer and rabbit anti-human polyclonal glial cell line-derived neurotrophic factor (GDNF) were applied to tissue sections and incubated at room

temperature according to the corresponding manufacture protocols for each antibody. After washing in phosphate buffer, sections were coated with secondary antibody (Biotinylated Goat Anti-Rabbit), conjugated with Streptavidin Horseradish Peroxidase Enzyme and visualized with 3,3′ diaminobenzidine (DAB) chromogene in DAB substrate. In all cases, negative control sections in which the primary antibodies not applied to tissue sections were carried out. Sections were dehydrated in ascending grades of ethanol, cleared in xylen and mounted with DPX mounting media. Sections were examined microscopically to evaluate the expressionTGFβ2 and GDNF in control at $7^{th}$ and $10^{th}$ days of incubation and in 50 µg/egg lanthanum-exposed embryos up to the $10^{th}$ day of incubation.

## RESULTS

### Toxicology

The preceding toxicity test revealed a consistent embryo resorption in lanthanum-exposed groups as compared to control. The percentage of embryo resorption was 15, 28 and 66% in 50, 100 and 150µg/egg of lanthanum-exposed, respectively. The resorped embryos were diminished in size and failed to organized as compared to the healthy embryos of lanthanum-exposed in the various doses and those of control (Pl. 1)

**Histological and immunohistochemical study:**

Eye

In 7 days-old of control embryos, well differentiated components of the eye were observed, retina, lens and cornea (Pl. 2 A). In 10 days-old of control embryos, folding of the retinal components (the neural and pigmented retina) was noted at the dorsal side of the developing lens forming the ciliary body (Pl. 2 B) as compared to the less differentiated ciliary body in lanthanum-exposed embryos of comparable age (Pl. 2 C). PAS-stained sections revealed faintly-stained polysaccharides at the periphery of the developing lens in both7 and 10 days-old of control embryos (Pl. 2 D, E). Less-stainability was observed in lanthanum-exposed embryos (Pl. 2 F) compared to control. In contrast, heavily-stained acid mucopolysaccharides were observed either in 7 or 10 days of control embryos throughout the developing components of the whole eye (Pl. 2 G, H). In lanthanum-exposed embryos less stainable acid mucopolysaccharides was detected (Pl. 2 I). Immunostaining of both the transforming growth factor (TGF β2) and the glial cell line-derived neurotrophic factor (GDNF) revealed the expression of both factors toward the inner surface of the neural epithelia either in 7 or 10 days-old control embryos (PL. 2 J, K, and M, N). Diminished expression of both factors in lanthanum-exposed embryos with concomitant deformed ciliary body was noted compared to control (Pl. 2 L, O).

Spinal cord

In 7 days-old of control embryos, the neural tube is regionally differentiated to form the white matter, gray matter and a silt-like central canal with its lining ependyme and basal plate (Pl. 3 A). At the $10^{th}$ day of development, a well differentiated horns of the gray matter were observed either in control or in lanthanum-exposed embryos (Pl. 3 b, C), respectively. Acid mucopolysaccharide staining revealed intense and homogenous distribution throughout the early differentiated white matter, gray matter and the ependyme of the neural tube of 7 days-old of control embryos (Pl. 3 D). At $10^{th}$ day of development, decrease of acid mucoplysachharides was noted throughout the differentiated white matter, gray matter and the ependyme cells (Pl. 3 E) as compared to that observed in 7 days-old of control embryos. Lanthanum exposure results in altered but homogenous stained acid mucopolysaccharides (Pl. 3 F) as compared to the control of comparable age. Immunostaining of both the transforming growth factor beta (TGF β2) and the glial cell line-derived neurotrophic factor (GDNF) revealed the contrast expression of both factors in control embryos at the $7^{th}$ and $10^{th}$ days of development. TGF β2 is sharply expressed at the $10^{th}$ day (Pl 3 H) as compared to the faint expression (Pl. 3 G) at the $7^{th}$ day of control embryos. In contrast, the expression of GDNF is best detected at the peripheral region of the differentiated neural tube (the white matter) at the $7^{th}$ day (Pl. 3 J) and then diminished at the $10^{th}$ day (Pl. 3 K) in control embryos. In lanthanum-exposed embryos of 10 days-old, diminished and patchy expression throughout developing spinal cord for TGF β2 (Pl. 3 I) and no observed expression of GDNF (Pl. 3 L) were noted. These observations were concomitant without alteration in the

fluorescence of acridin orange-stained sections of spinal cord of control (Pl. 3 M) or lanthanum-exposed embryos (Pl. 3 N) of 10 days-old.

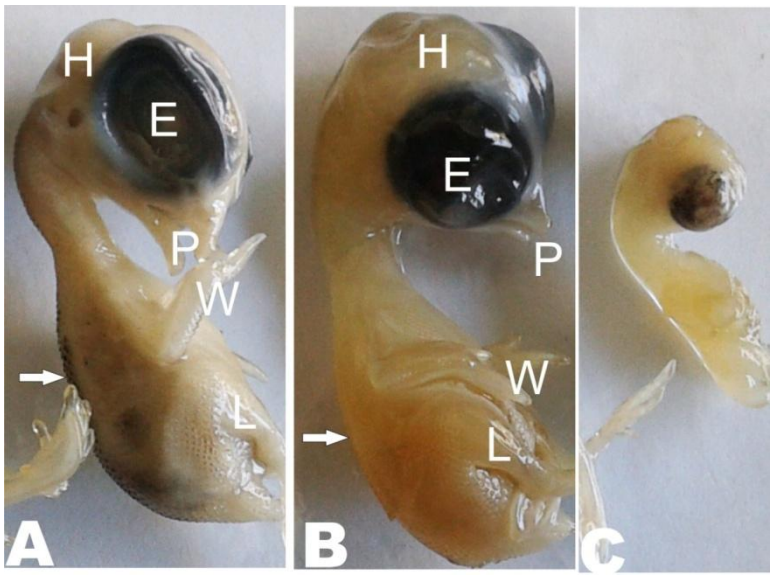
Lung

In 7 days-old embryos, alveolated proximal and distal mesenchymal regions of the developing lung were differentiated (Pl. 4 A). By 10 days of incubation, both of the proximal and distal regions were alveolated either in the control (Pl. 4 B) or in lanthanum-exposed embryos (Pl. 4 C). Morphogenesis of alveoli is accompanied with the increase of acid mucopolysaccharides in either the alveolar epithelia or in the stroma of the developing lung from 7 – 10 days old of control embryos (Pl. 4 D, E), respectively. Decrease of acid mucopolysacchrides was noted in both the epithelial and mesenchymal components of the developing lung (Pl. 4 F) as compared to the control embryos of comparable age. Expression of both TGFβ2 (Pl. 4 G, H) and GDNF (Pl. 4 J, K) in the developing lung is increased in both the epithelium and mesenchyme components from 7 – 10 days-old of control embryos and inhibited by lanthanum exposure (Pl. 4 I, L).

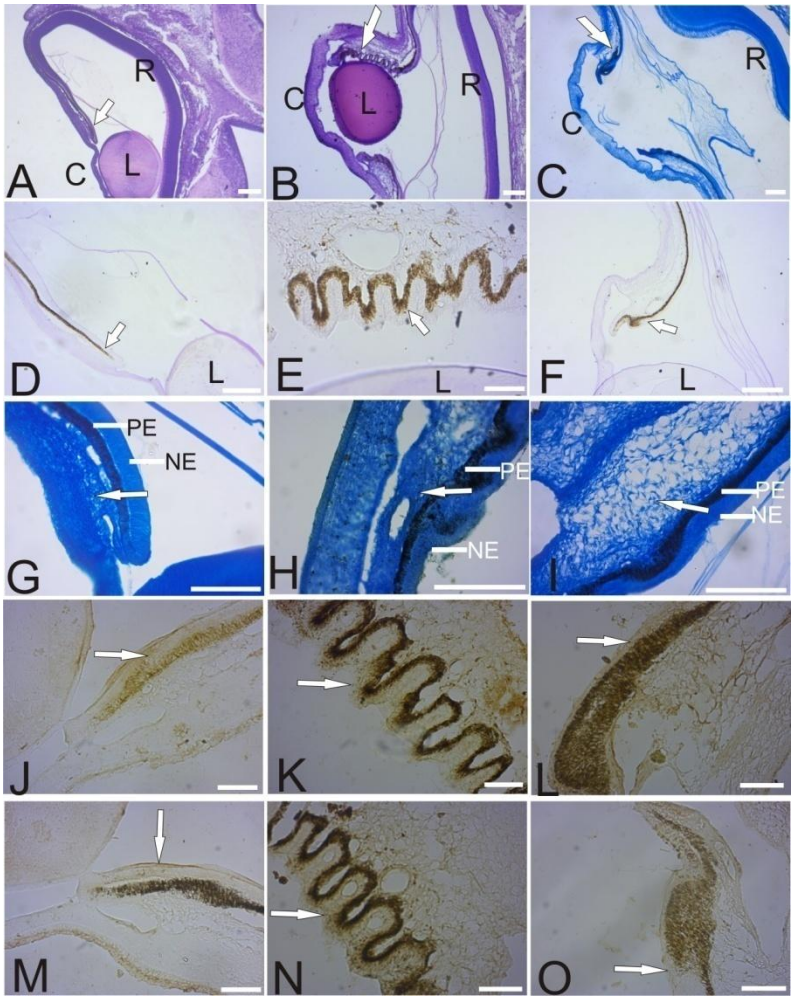
Liver and kidney

In either the 7$^{th}$ or the 10$^{th}$ day of development, the liver has the definite architecture in which anastomosing hepatic cords are dispersed in a meshwork of blood sinusoids (Pl. 5 A). PAS staining technique revealed a negative reaction (Pl. 5 B) while acridine orange staining revealed shining fluorescence of nuclei in either the hepatic cells or those of blood cells (Pl. 5 C). In lanthanum-exposed embryos the same histological architecture was found except the altered staining of nuclei in either haematoxylin or PAS reaction which appeared black as compared to control (Pl. 5 D, E). Also, in lanthanum exposed embryos shining of fluorescence was inhibited throughout the hepatic parenchyma (Pl. 5 F). The effect of lanthanum on the developed mesonephric kidney is consistent with that observed in the liver. Altered nuclear staining (Pl. 5 J, K) and decrease of fluorescence (Pl 5 L) were noted compared to control (Pl. 5 G – I).

Immunohistochemical detection of TGFβ2 revealed an intense expression throughout the liver parenchyma and kidney tubules at the 7$^{th}$ day of incubation (Pl. 6 A, G). Decrease of TGFβ2 expression was noted at 10$^{th}$ day in both tissues (Pl. 6 B, H) and inhibited in lanthanum-exposed embryos (Pl. 6 C, I). In contrast, the expression of GDNF was increased from 7-10 days of incubation in both the liver (Pl. 6D, E) and kidney (Pl. 6 J, K). In lanthanum-exposed embryos, inhibition of GDNF expression was also noted (Pl. F, L) with concomitant altered stained nuclei in both tissues similar to that observed in both TGFβ2-immunostained and at the histological level.

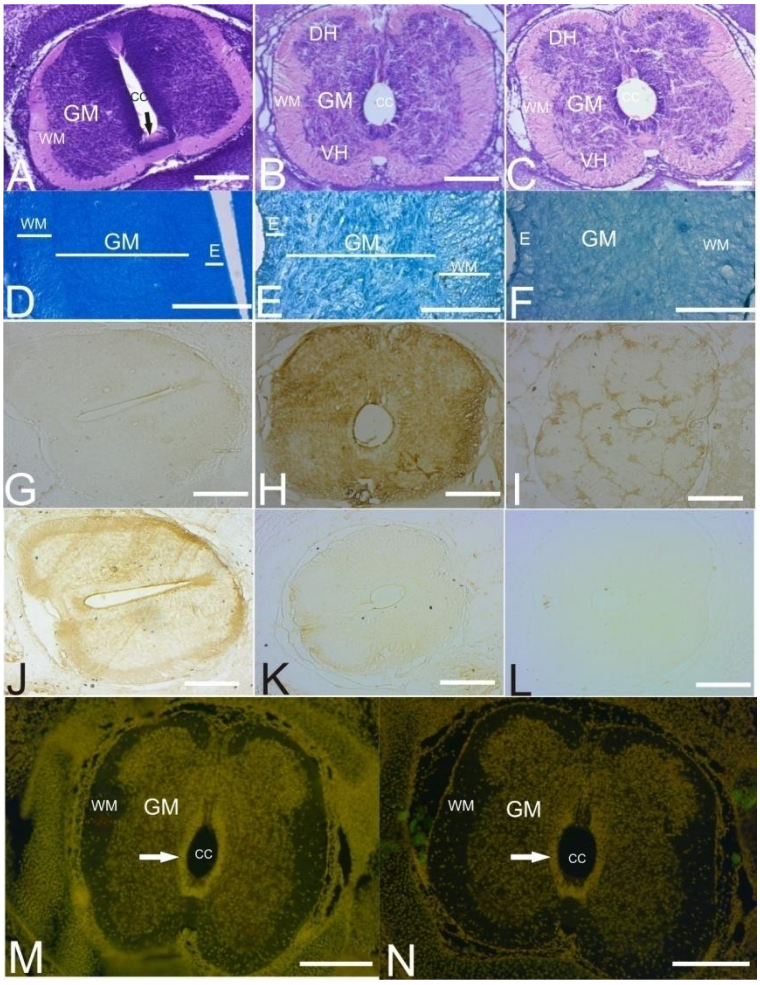


**Pl. 1:** Photographs of 10 days-old embryos of control (**A**), healthy embryos of lanthanum-exposed in either 50, 100, 150µg/egg (**B**) against the resorped embryos (**C**) in various lanthanum concentrations. Head(H), eyes (E), peak (P) , wings (W), legs (L) and featherless skin was noted in healthy embryos of lanthanum-exposed compared to control (arrows). X 0.8

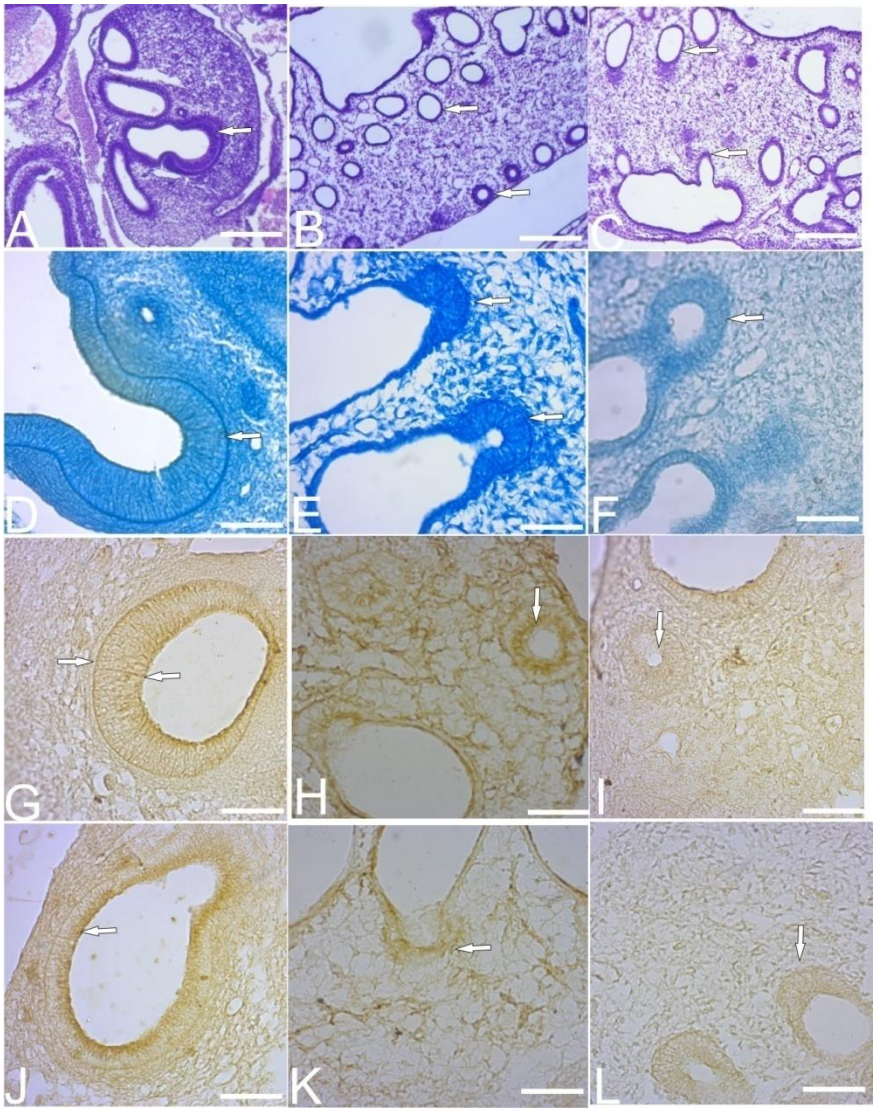



**2: A-C:** Sections of the developing eye at 7th day (**A**), 10th day of control (**B**) and 10th day of lanthanum-exposed embryos (**C**). Lens (L), retina (R), cornea (C) and the differentiated ciliary body from 7 – 10 days (arrows) of control compared to the less differentiated ciliary body in lanthanum-exposed embryos (arrow) are shown. **H&E stain, scale bar 10 µm. D – F:** Sections at7th day (**D**), 10th day (**E**) of control and 10th day of lanthanum-exposed embryos (**F**) showing polysachhrides at the periphery of the developing lens (L). Arrested morphogenesis of ciliary body in lanthanum-exposed embryos was noted compared to the full differentiated in control (arrows). **PAS stain, scale bar 50 µm. G – I:** Intense acid mucopolysaccharide staining at 7th day (**G**), 10th day (**H**) of control versus the decreased stainability in lanthanum-exposed embryos (I) of 10 days old (arrows). Folding of both the pigmented (PE) and retinal epithelia (RE) in control embryos compared to the less differentiated ones in lanthanum-exposed embryos were noted.
**Alcian blue stain, scale bar 50 µm. J – O:** Positive immunostained sections of TGFβ2 at 7th (**J**), 10th (**K**) days of control embryos toward the inner surface of the neural epithelia of the primordial and full developed ciliary body (arrows). Diminished expression (arrow) with deformed ciliary body was noted in lanthanum-

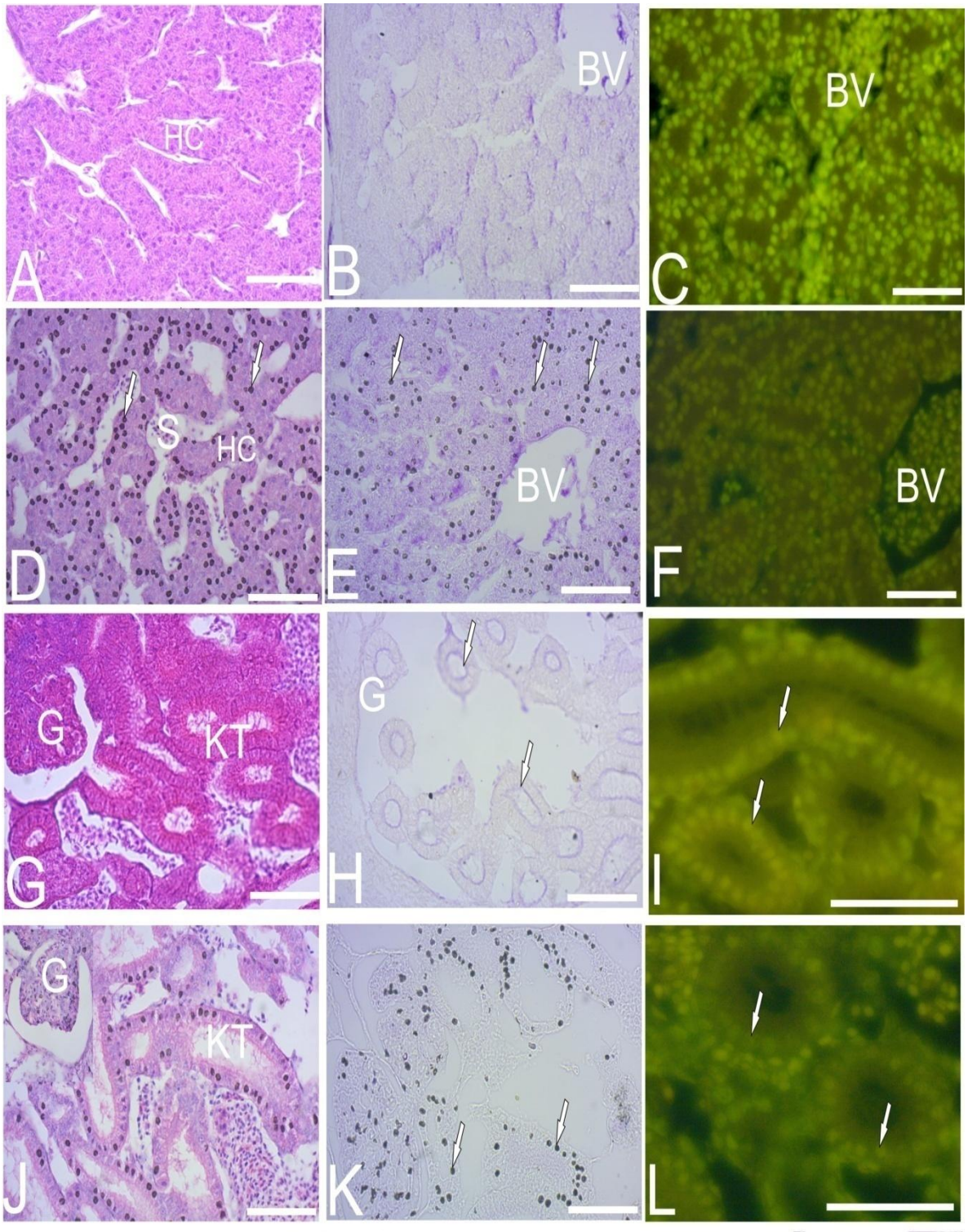
exposed embryos (**L**). GDNF- immunostained sections showing a similar expression (arrows) at 7th (**M**), 10th (**N**) or in lanthanum-exposed embryos (**O**). **Scale bar 50 µm.**



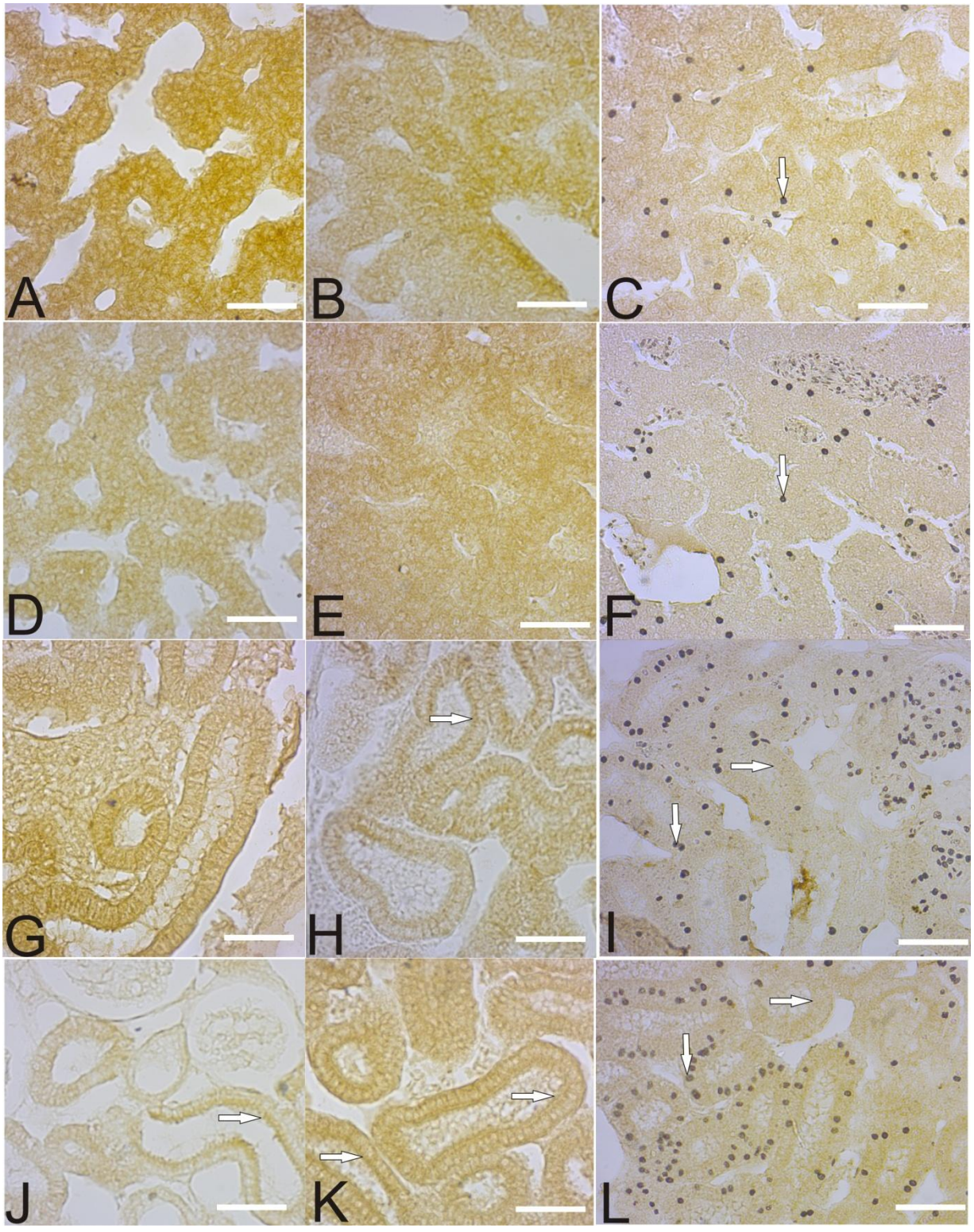

**PL. 3: A – C:** Section of the differentiated neural tube of 7 days-old of control embryos (**A**). **B, C:** Sections of spinal cord at 10th day of control and lanthanum-exposed, respectively. White matter (WM), gray matter (GM), silt-like central canal (CC), the basal plate (arrow), dorsal horn (DH) and the ventral horn (VH) are shown. **H&E stain, scale bar 10 µm. D - F:** High magnified field of the differentiated neural tube of 7 days-old of control embryos (**D**) and high magnified fields of spinal cord of control (**E**) and lanthanum-exposed (**F**) at 10th day of development, respectively, showing intense stained acid mucopolysaccharide at 7th day that decreased at the 10th day of control and altered in lanthanum-exposed throughout the differentiated white matter (WM), gray matter (GM) and in the ependyme cells (E). **Alcian blue stain, scale bar 50 µm. G – L:** TGFβ2-immunostained sections showing increased expression throughout the developing spinal cord from 7 (G) to 10 (**H**) days-old of control embryos compared to the patchy expression in lanthanum-exposed embryos (**I**) of 10 days-old and GDNF-immunostained sections showing the earlier expression at the periphery of the differentiated neural tube of 7days-old (**J**) of control embryos, faint expression in 10 days-old of control (**K**) against the diminished expression in lanthanum-exposed embryos (**L**) of 10 days-old.**Scale bar 10 µm. M, N:** Photomicrograph of transverse sections of spinal cord of control (M) and lanthanum-exposed (N) of 10 days-old, respectively, showing similar fluorescence of white matter (WM), gray matter (GM) and the ependyme (arrow) surround the central canal (CC) of spinal cord**. Acridin orange stain, scale bar 20 µm.**




**Pl. 4: A-C:** Sections of the developing lung in 7 days- (**A**), 10 days-old (**B**) of control embryos and 10 days-old of lanthanum-exposed embryos (**C**), respectively, showing the developing alveoli (arrows) in a background mesenchyme. **H&E stain, scale bar 10 µm.** Increased acid mucopolysaccharides in alveolar epithelia of control embryos from 7$^{th}$ (**D**) to the 10$^{th}$ day (**E**) of control embryos against the decrease in lanthanum-exposed embryos (**F**) (Arrows). **Alcian blue stain scale bar 50 µm. G – L:** TGFβ2-immunostained sections of lung showing increased expression in stroma and epithelia (arrows) of the developing lung in 7$^{th}$ (**G**) and 10$^{th}$ (**H**) day of control embryos that inhibited by lanthanum (**I**) in both the alveoli (arrow) and stroma and GDNF-immunostained showing increased expression in stroma and epithelia (arrows) of the developing lung in 7$^{th}$ (**J**) and 10$^{th}$ (**K**) day of control embryos that inhibited by lanthanum (**L**) in both the alveoli (arrow) and stroma. **Scale bar 50 µm.**




**l. 5: A – F:** H&E-, PAS- and acridin orange-stained liver sections of control (**A-C**) and lanthanum-exposed (**D – F**) showing hepatic cords (HC), blood vessels(BV) sinusoids (S), altered stained nuclei (arrows) and depressed flourescense in lanthanum-exposed compared to control. **G –L:** H&E-, PAS- and acridin orange-stained mesonephros of control (**G - I**) and lanthanum-exposed (**J - L**) showing the developing glomeruli (G), kidney tubules (KT), altered stained nuclei in PAS and slight depression of acridin orange fluorescence of cell nuclei (arrows) of lanthanum-exposed compared to control. **Scale bar 10µm.**




**Pl. 6:** Expression of TGFβ2- (A – C) and GDNF- (D – F) immunostained liver sections of control from 7 (A, D) to 10 days-old (B, E) and inhibition in lanthanum-exposed (C,F) with altered stained nuclei (arrow). TGFβ2- (G - I) and GDNF- (J– L) immunostained mesonephros of control from 7 (G, J) to 10 days-old (H, K) that

concentrated to the brush border of tubules (arrows) and inhibition in lanthanum-exposed (I, L) with altered stained nuclei (arrows). **Scale bar 10µm.**

## DISCUSSION

The present investigation revealed a concentration-dependent embryo deformation at the level of morphological observation. In normal embryos, polysaccharides and the studied growth factors are best expressed at the $7^{th}$ and the $10^{th}$ day of embryonic age in the studied organs. In the lowest dose of lanthanum-exposed embryos, altered nuclear staining at both histological and immunohistochemical, decrease of extracellular matrix, inhibition of the growth factors in the developing ciliary body, spinal cord, lung, liver and kidney was observed compared to control. In this context, the effect of lanthanum has been studied extensively in different animal tissues, both to establish the normal function and determine dosage for therapeutic uses. In mammals, a single intraperitoneal injection of 44mg/kg b.w into pregnant mice prior to preimplantation was found to reduce the number of successful pregnancies and the average of litter size (Abramzuck, 1985). Lanthanum cations are well known for their inhibitory effect on calcium channels. As competitive antagonist of calcium, lanthanum cations was found to prevent the movement of calcium into the cell that result in altered neurobehaviour in mammals and chicks (Briner et al., 2000; Feng, et al., 2006; Che et al., 2011; Yang et al., 2013). Lanthanum was profoundly inhibit in vitro important cellular response in which calcium plays a significant role which give us a good interpretation for the observed developmental arrest and embryo resorption at the various doses of lanthanum used in the present investigation.

Concerning the altered staining of nuclei at both histological and immunohistochemical levels, lanthanum was found to reacts in vitro with various tissue components, like nucleoproteins, plasma proteins, amino acids, phospholipids, enzymes, intermediary metabolites and inorganic phosphate. Lanthanum affinity towards phosphate is high and leads to the formation of insoluble complexes with phosphatases, cleaving of phosphate groups from ATP, glycerophosphate, nucleotides and nucleic acids. Lanthanum was found also to precipitate DNA in vitro from aqueous solution by combining with the phosphate groups of adjacent chains. These data confirms the altered staining of nuclei at both histological and immunohistochemical investigation in the present study and the principles of therapeutic uses of lanthanum in hyperphosphatimia in chronic kidney diseases as reported by Shigematsu et al., (2012) and Vegter et al., (2012).

From the other hand, inhibition of extracellular matrix as demonstrated with PAS and alcian blue stains during the differentiation of ciliary body of the developing eye and with alcian blue during the differentiation of the neural tube and early ramification of lung alveoli in lanthanum-exposed embryos at $10^{th}$ was noted. These extracellular matrix are known not only binds the cells together but also influences their survival, development, shape, polarity, and behavior. In general, the extracellular matrix contains fibrillar proteins (e.g., collagens), glycoproteins (e.g., laminins, fibronectin, tenascins), and several classes of proteoglycans (heparan sulfate-, chondroitin sulfate-, dermatan sulfate-, and keratan sulfate proteoglycans). The latter mainly consist of large glycosaminoglycan (GAG) chains, covalently linked to extracellular or membrane bound core proteins. In this context, it was suggest that $LaCl_3$ reduce the extracellular matrix formation at certain concentrations during wound healing as an anti-fibrous agent in clinical treatment of severe burn (Dai et al., 2006). Of our opinion, suppression (as noted in the differentiation of ciliary body and ramification of lung) or denaturation (as noted in the differentiation of spinal cord) of the extracellular matrix in lanthanum-exposed embryos may led to improper development by the virtue of its binding to cell nuclei (mentioned above) that may result in altered biosynthetic activity and consequently affecting cell binding, migration or signal transduction between different cells.

Coincides with the depressed staining of the extracellular matrix, both of the growth factors (TGF β2 and GDNF) are down regulated in lanthanum-exposed embryos in the studied organs. This down regulation was accompanied with altered staining of nuclei as noted in both of the liver and kidney immuno-stained sections. Transforming growth factors are a large family of cytokines that controls many aspects of cellular function, including cellular proliferation, differentiation, migration, apoptosis, adhesion, angiogenesis, immune surveillance, and survival. In this context, blocking TGF-beta was found to prevent primary or metastatic tumors from seeding and reseeding. In osteolytic bone metastasis, blocking TGF-beta interrupt the cycle of TGF-beta-induced osteoclastogenic factors and halt tumor growth (Loeys et al., 2006). Also, signaling via (TGFβ1) was differentially affects the rates of branching and growth of the airways in the embryonic chicken lung (Gleghorn et al., 2012). In addition, GDNF as a distinct member of the transforming growth factor-beta superfamily (Roberts and Sporn, 1990)

was early expressed in white matter than TGFβ2 during the differentiated neural tube in present study. This early expression can be attributed to the functional activity of glial cells since astrocytes were found to be the major source for GDNF upon brain injury (Bresjanac and Antauer, 2000**)** and exerts a strictly local expression of GDNF (Drinkut et al., 2012**)**. It was reported that GDNF support the survival and regulate the differentiation of many peripheral neurons, including sympathetic, parasympathetic, sensory and enteric neurons (Rrind et al., 2005). The studied growth factors were also heavily expressed in the differentiated liver and kidney during the earlier stage of development and inhibited with lanthanum exposure. Similar intense expression was reported in normal development of the kidney in rodents (Suter-Crazzolara and Unsicker, 1994) and modulation of renal blood vessel formation (Kloth and Suter-Crazzolara, 2000).The importance of the studied growth factors in normal development was recorded. TGFbeta2 knockout mice or deletion of its receptor results in multiple developmental defects and disruption of lung epithelial morphogenesis (Sanford et al., 1997; Li et al. 2008) and renal agenesis was reported for GDNF in knockout mice (Cullen-McEwen et al., 2001).

In conclusion, the present investigation revealed in addition to lanthanum toxicity in early developmental period (fertilized eggs) of embryogenesis. It also affect the cellular proliferation as observed in the developing ciliary body of the developing eye, extracellular matrix and the expression of TGFβ2 and GDNF as members of transforming growth factor responsible for angiogenesis in different organs. The study revealed also that beside the therapeutic role of lanthanum in hyperphosphatemia, the growth factors in this investigation by its virtue of their angiogenic activity may contribute to the advent of a new generation of agents that directly affect tumor seeding and reseeding (metastasis) through lanthanum therapeutic uses.

## References

Abramczuk, J. (1985).The effects of lanthanum chloride on pregnancy in mice and on preimplantation mouse embryos in vitro. J. Toxicol., 34 (4): 315-320.

Bresjanac, M. and Antauer, G. (2000). Reactive Astrocytes of the Quinolinic Acid-Lesioned Rat Striatum Express GFR alpha1 as Well as GDNF in vivo. Exp. Neurol., 164:53-59.

Briner, W.; Rycek, R. F.; Moellenberndt, A. and Dannull, K. (2000). Neuro-developmental effects of lanthanum in mice. Neurotoxicol. Teratol., 22: 573 – 581.

Buchlowalow, B. I. and Bocker, W.( 2010). Immunohistochemistry. Basics and methods. Springer Verlag Berlin Heidelberg, PP: 48.

Che, Y.; Xing, R.; Zhu, Y.; Cui, Y.; Jiang, X. (2011). Effects of Lanthanum Chloride Administration on Detouring Learning in Chicks. Biological Trace Element Research, 143, (1): 274-280 .

Cullen-McEwen, L. A; Drago, J. and Bertram, J. F. (2001). Nephron endowment in glial cell line-derived neurotrophic factor (GDNF) heterozygous mice. Kidney International, **60**, 31–36.

Dai, Y.; Zhong, X.; Dai. G. Li. J.; Li, J.; Wen, Z.; Cao, Y. and Li, G. (2006). Inhibitory effects of lanthanum chloride on extracellular matrix in injury tissues of rats. Burns, 32 (7): 858 – 866.

Das, T.; Sharma, A.; Talukder, G. (1988). Effects of lanthanum on cellular systems, a review. Biol. Trace Elem. Res., 18: 201 – 228.

Drinkut, A.; Tereshchenko Y.; Schulz, J. B.; Bahr, M. and, Kugler, S. (2012). Efficient gene therapy for Parkinson's disease using astrocytes as hosts for localized neurotrophic factor delivery. Mol Ther **20**: 534–543

Drury, R. A. B. and Wallington, E. A. (1976). Carleton's Histological Technique. Oxford University Press, London.

Fan, G. Q.; Yuan, Z. K.; Zheng, H. L. and Liu, Z. J. (2004).Study on the effect of exposure to rare earth elements and health-responses in children aged 7 – 10 years. J. Hyg. Res., 33: 23 – 28.

Feng , L.; Xiao , H.; He , X.; Li, Z.; Le, F.; Liu, N.; Chi, Z.; Zhao , Y. and Zhang, Z. (2006). Long-term effects of lanthanum intake on the neurobehavioral development of the rat. Neurotoxicol. Teratol., 28 (1): 119 – 124.

Folkman , J. Klagsbrun, M. (1987). Angiogenic Factors. Science, 235(4787): 442 – 447.

Folkman, J. (1990). What is the evidence that tumors are angiogenesis dependent? J. Natl. Cancer Inst., **82:** 4-6.

Gleghorn, J. P.; Kwak, J.; Pavlovich, A. L. and Nelson, C. M. (2012). Inhibitory Morphogens and Monopodial Branching of the Embryonic Chicken Lung. Developmentl Dynamics, 241:852–862.

Guo, B. S.; Zhu, W. M. and Xiong, B. K. (1990). Rare earths in agriculture. China Agricultureal Science and Technology Press, Beijig, P:11.

Guo, X. B.; Gao, Z. H.; Luo, L. Z. and Yao, B. Y. (2000). Effects of rare earth compounds on metabolic cooperation between Chinese hamster V79 cell. Zhonghua Yufang Yixue Zazhi, 34: 17-19.
Kloth, C. and Suter-Crazzolara, C. (2000). Modulation of Renal Blood Vessel Formation by GlialCell Line-Derived Neurotrophic Factor. Microvascular Research, 59, 190–194.
Li, M.; Li, C.; Liu, Y. H.; Xing, Y.; Hu, L.; Borok, Z.; Kwong, K. Y. And Minoo, P. ( 2008). Mesodermal deletion of transforming growth factor-beta receptor II disrupts lung epithelial morphogenesis: cross-talk between TGF-beta and Sonic hedgehog pathways. J. Biol. Chem., 283(52):36257–36264.
Li, X. Q.; Jiang, J. J.; Yang, L.; Liu, M. and Yang, L. Y. (2000). Effect of low level mixed rare earth Changle on learning and memory, locomotor activity and NMDA-and M-receptors activity and morphology of divisions of hippocampal cortex in rats. Zhonghua Yufang. Yixue. Zazhi., 34: 20-23.
Li, X.; He, P.; Xia, J.; Song, S.; Lu, J.; Liu, Y. (2009). Effect of lanthanum chloride on growth of breast cancer cells and regulation of c-met transcription. Frontiers of Medicine in China, 3(3): 336-340.
Liu, J. M.; Chen, D.; Wang, X. M.; Nie, Y. X.; Li, Y. and Li, J. (2000). Long-term effect of Changle and $La(NO_3)_3$ on level of IL-2 and g-IFN of splenic lymphocytes in rats. Zhonghua. Yufang. Yixue. Zazhi., 34:49-51.
Loeys, B. L.; Schwarze, U.; Holm, T.; Callewaert, B. L.; Thomas, G. H. et al., (2006). Aneurysm syndromes caused by mutations in the TGF-beta receptor. N. Engl. J. Med., 355(8):788-98.
Nakamura, Y.; Tsumura, Y.; Tonogai, Y.; Shibata, T. and Ito, Y. (1997). Differences in behavior among the chlorides of seven rare earth elements administered intravenously to rats. Fundam. Appl. Toxicol., 37: 106-116.
Rind, H. B.; Butowt, R.;, von Bartheld, C. S. (2005): Synaptic targeting of retrogradely transported trophic factors in motoneurons: comparison of glial cell line-derived neurotrophic factor, brain-derived neurotrophic factor, and cardiotrophin-1 with tetanus toxin. J. Neurosci., 25(3):539- 549.
Robert, A. B. and Sporn, M. B. (1990) The transforming growth factors-β. In Sporn,M.B. and Roberts,A.B. (eds), Handbook of Experimental Pharmacology. Peptide Growth Factors and Their Receptors. Springer, Berlin, pp. 419–472.
Rosen, L. S. (2002). Clinical experience with angiogenesis signaling inhibitors: focus on vascular endothelial growth factor (VEGF) blockers. Cancer Control, 9:36–44.

Sanford ,L .P.; Ormsby I.; de Gittenberger, G. A.; Sariola, H.; Friedman, R.; Boivin, G.P.; Cardell, E.L. and Doetschman, T. (1997). TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. Development, 124(13):2659–2670.

Shigematsu, T.; Nakashima, Y.; Ohya, M.; Tatsuta, K.; Koreeda, D.; Yoshimoto, W.; Yamanaka, S.; Sakaguchi, T.; Hanba, Y.; Mima, T. and Negi, S. (2012). The management of hyperphosphatemia by lanthanum carbonate in chronic kidney disease patients . International J. Nephrol. Renovascular Disease, 5: 81 – 89.
Suter-Crazzolara, C., and Unsicker, K. (1994). GDNF is expressed in two forms in many tissues outside the CNS. NeuroReport **5,** 2486–24874.
Vegter, S.; Tolley, K.; Keith, M. S.; Lok, C. E.; Soroka, S. D.; Morton, A. R.(2012). Cost-effectiveness of lanthanum carbonate in the treatment of hyperphosphatemia in dialysis patients: a Canadian payer perspective. Clinical Therapeutics, 34(7):1531-1543.
Xu, H. E.; Gao, G. H.; Jia, F. L.; Wangh, X. Y.; Xie, Q.; Liu, H. S.and Wang, N. F. (2000). Effect of mixed rare earth Changle on the micronuclei formation of blood lymphocytes in rats. Zhonghua Yufang Yixue Zazhi ; 34: 5-7.
Yang, J.; Liu, Q.; Wu, S.; Xi,Q. and Cai,Y. (2013). Effects of lanthanum chloride on glutamate level, intracellular calcium concentration and caspases expression in the rat hippocampus. Biometals, 26 (1): 43-59.
Zhu, W. F.; Xu, S. Q.; Shao, P. P.; Zhang, H.; Wu, D. S.; Yang, W. J. and Feng, J. (1997). Bioelectrical activity of the central nervous system among population in a rare earth element area. Biol. Trace Elem. Res., 57: 71 – 77.