Prenatal and Postnatal Effects Of Curcumin and Vit C on Lead Acetate induced Alterations in Developing Kidneys of Albino Rat

Saadia Ahmed Shalaby, Essam Mohammed Eid, Omar Abd El Aziz Allam, Osama Fouad Ahmed and Nehal Fahmy Shaheen

Anatomy and Embryology Department, Benha Faculty of Medicine, Benha university

shaheenezz@yahoo.com

Introduction: poisoning with lead between pregnant women being an important public health problem, as it disturbs development. As lead can cross the placenta accordingly; intrauterine lead poisoning harmful to the woman and also to the developing baby. Curcumin has been shown to improve oxidative stress and it is considered to be a potent antioxidant. Vitamin C, is a chelating agent, have the capability to protect cells from oxidative stress. Aim of Work : is to Study of the histological and ultrastructural changes in the kidney of rat pups at 18th day of gestation and at weaning after oral intake of lead acetate to the pregnant rats from the first day of pregnancy till weaning and the protective role of curcumin and vitamin C. Materials and methods : 12 adult female albino rats allowed to be pregnant and they divided into: control group and experimential group. The control group consisted of 4 adult female albino rats. Also the experimential group divided into two groups each group contain 4 adult female albino rats (Lead acetate treated group and Lead acetate plus curcumin & vit C). At 18th day of gestation Two pregnant rats from each group of control and experimental subgroups were anaesthetized, the abdomen and uterus was opened and the fetuses were extracted then the abdomen of each fetus was opened and the kidney was extracted. the outcome of the remaining 2 pregnant rats from each group will be sacrificed after weaning. Results: the lead acetate treated groups (prenatal and postnatal) showed discoumation of cells of the proximal tubules, the distal tubule are degenerated. The glomeruli are intact. While In lead acetate plus curcumin and vitamin C treated group the glomeruli are intact but its renal space is wide, the proximal and distal tubules appear like normal. Conclusions: Lead acetate affects the kidney during gestation and lactation periods. Adminstration of curcumin and vitamin C minimize the harmful effect of lead acetate on developing kidneys.

Keywords : Prenatal, Postnatal, Curcumin, Lead acetate, vit C, Developing Kidneys.

1. Introduction:

Lead is known a widespread environmental pollutant due to its important role in current industry. However it is being a serious problem in many developing and industrializing countries for occupational and environmental exposures [1]. Lead can cross the placenta freely so gestational lead exposure is harmful to the woman and to the developing fetus [2]. Lead enhancing lipid peroxidation product in kidney tissue so produces oxidative damage in the kidney [3].Curcumin is a biologically active compound, which can improve oxidative stress and so considered as a powerful antioxidant [4]. In lead-exposed rats, vitamin C has the ability to bound lead, like that of EDTA. It may increase urinary removal of lead and reduce its hepatic and renal load [5].
2. Materials and methods:

A) Materials:

Animals:

Twelve adult female albino rats, ranged in weight between 200-250 gm each will be used in this study. The female rats will be placed in quarantine for one week prior to breeding housed in plastic cages in a controlled environment at 23-25 °C, a 12 hour light- dark cycle, and free access to water and balanced diet. After acclimatization to laboratory conditions two females will be kept overnight with one male to allow matting the presence of spermatozoa in the vaginal smear in the next morning indicator as day zero of pregnancy. The pregnant rats will be housed on individual cages, to be weighed weekly, and their general condition will be noticed.

Drugs used:

1-Lead acetate: is purchased from Sigma- Aldrich company. Freshly dissolved in distilled water and administrated orally by gastric intubations in a dose 10mg/Kg/ day one daily. 2- Curcumin: is purchased from Sigma- Aldrich company. It appears bright yellow to orange color powder. one gm of curcumin will be dissolved in 100 ml ethyl oleat, also purchased from Sigma- Aldrich company, so each 1ml ethyl oleat contains 10 mg. Each pregnant female rat were received 100mg curcumin /Kg/ day, administrated orally by gastric intubations once daily. 3-Vitamin C: is obtained from El – Gomhoria chemical company in the form of white powder soluble in water. One gm of vit C will be dissolved in 100 ml distilled water, so each 1 ml of solution contains 10 mg vit C. according to weight of rat, Each pregnant female rat were received 140mg/Kg/ day, administrated orally by gastric intubations once daily.

B) Methods:

The pregnant rats will be divided into two main groups: control group and experimental groups. Control group: Consists of four pregnant rats. Each one will receive a single oral dose distilled water from the first day of pregnancy till the end of lactating period. Experimental groups: will be divided into two subgroups. 1- (Lead acetate treated group) Consists of four pregnant rats. Each one will receive a single oral dose of lead acetate solution from the first day of pregnancy till the end of lactating period. 2- (Lead acetate plus curcumin & vit C) Consists of 4 pregnant rats. Each one will receive a single oral daily dose of lead acetate solution, curcumin solution and vit C solution/ once /day from the first day of pregnancy till the end of lactating period. At 18th day of gestation Two pregnant rats from each group of control and experimental subgroups were anaesthetized using ether inhalation, the abdomen and uterus was opened and the fetuses were extracted then the abdomen of each fetus was opened and the kidney was extracted. the outcome of the remaining 2 pregnant rats from each group will be sacrificed after weaning. The abdomen of each offspring was opened and the kidney was extracted.

Specimen and tissue preparation:

The kidneys of these subgroups will be prepared for light and electron microscopic analysis. The kidney specimens for light microscopic analysis will be stained by haematoxylin and eosin and Masson’s trichrom stain.

RESULTS:

A- (Fetal rats aged 18 –days ) 1- Control group: By light microscopey, the renal cortex of the fetal rat aged 18 days show subcapsular zone containing the immature forms of renal developmental stages in the form of renal vesicle, comma shaped, S shaped bodies and immature glomerulus which appears as dense rounded structure. The proximal tubule are lined by simple cuboidal epithelium with rounded, basal, vesicular nuclei. The distal tubules have a wide lumen and are lined by low cuboidal cells with central spherical nuclei. Fig.( 1-A) in Masson’s Trichrome stain, the connective tissue is minimal in amount. Fig. (1- B) . By electron microscopy: The cells lining the proximal
convoluted tubule have a single rounded nuclei. Its cytoplasm contains many mitochondria. These cells have many microvilli at its apical surfaces. Its lumen is obliterated by microvilli Fig. (1-C).

2-Treated groups: a-Lead acetate treated group: By light microscopy, the proximal tubules are dilated and the lining epithelium is flattened with discomium of cells in its lumen. some distal tubule are degenerated. The glomeruli are intact. Fig.(2-A) In Masson's Trichrome stain, the connective tissue are increased in amount around the glomerulus and the tubules. Fig(2-B). By electron microscopy, The cells lining the proximal tubule show vacuolated mitochondria , their microvilli are few and the lumen is wide. Fig. (2-C). b- Lead acetate plus curcumin and vitamin C treated group: By light microscopy, the proximal tube ,the distal tube and the glomerulus appear like normal Fig. (3-A). Minimal amount of connective tissue are seen between the glomeruli and the tubule in Masson's Trichrome stain Fig. (3-B). By electron microscopy , The cell lining the proximal tubule is cuboidal in shape. It has rounded nucleus and elongated mitochondria. The microvilli are condensed and project in its lumen. Fig. (3-C)

B) Fetal rats at weaning:

1-Control group: By light microscopy , the mature glomeruli have parietal layer, visceral layer and renal space in between . The proximal tubule consists of columnar cells with rounded basal nuclei .The distal tubules have low cubical cells with wide lumen Fig. (4-A). In Masson’s Trichrome stain, the connective tissue is minimal in amount around the glomeruli and tubules Fig. (4-B). By electron microscopy, The proximal tubule are lined with columnar cells which have large rounded nuclei, apical microvilli . The cytoplasm contains elongated mitochondria Fig. (4-C)

2-Treated groups: A-Lead acetate treated group: By light microscopy, the glomeruli are degenerated with wide renal space when compared with control group.The proximal and distal tubules are also degenerated Fig. (5-A). In Masson’s Trichrome stain, there is condensed connective tissue around the glomerulus and the proximal and distal tubules Fig. (5-B). By electron microscopy, The cells lining the proximal convoluted tubule show apical multiple vacuoles and cytoplasmic extension towards the lumen. The groups of mitochondria are intact Fig. (5-C). B- Lead acetate plus curcumin and vitamin C treated group : By light microscopy, the glomeruli are intact but its renal space is wide , the proximal and distal tubules appear like normal Fig. (6-A). In Masson’s Trichrome stain, there is condensed connective tissue around the glomerulus and the proximal and distal tubules Fig. (6-B). By electron microscopy, The cells lining the proximal tubule are columnar with large oval nucleus.The cytoplasm contain elongated mitochondria and few vacuoles. Fig. (6-C).

DISCUSSION:

In the present study the renal cortex of control fetal rat aged 18 days showed the subcapsular zone containing immature forms of renal developmental stages in the form of renal vesicle, comma shaped , S shaped bodies and immature glomerulus which appeared as a dense rounded structure . The proximal tubules were lined by simple cuboidal epithelium with rounded, basal, vesicular nuclei . The distal tubules had a wide lumen and were lined by low cuboidal cells with central spherical nuclei. This is in agreement with Marquez et al., (2002) who reported that the developmental stages of the nephron are renal vesicles, comma- and S-shaped bodies can be visualized until the 5th day postnatal. By Haematoxylin and Eosin stain, the renal cortex of control rats at weaning show mature glomeruli that have parietal layer, visceral layer and renal space in between . The proximal tubule consists of columnar cells with rounded basal nuclei .The distal tubules have low cubical cells with wide lumen. This is in agreement with Mohamed and Saleh,( 2010) who found that The kidney cortex was formed of renal corpuscles, proximal and distal convoluted tubules. Each renal corpuscle consisted of a glomerular capillary tuft surrounded by Bowman’s capsule. The capsule consisted of
parietal layer formed of simple squamous epithelium and a visceral layer enveloping the capillaries of the glomerulus. The renal space present between the two layers of the capsule. The distal tubule had wider lumen and less acidophilic cytoplasm than the proximal tubules. In this study, the administration of lead acetate to the pregnant rats in a dose 10mg/Kg/day orally leads to changes observed by Haematoxylin and Eosin stained sections where the proximal tubules appeared dilated and the lining epithelium was flattened with discoumation of cells in its lumen. Some distal tubule were degenerated. The glomeruli were intact at fetal age 18, but the glomeruli appeared degenerated with wide renal space at weaning. This in agreement with Mohamed and Saleh,(2010) who reported that treatment with lead acetate disrupted the normal histological structure of the kidney. There was degeneration in the form of increase in the size of the renal corpuscles, thickening in the glomerular basement membrane and the glomerulus showed increased renal space. There was also dilated congested blood vessels in the interstitial space. Increase in the size of proximal and distal tubules with vacuolation and shedding of the lining epithelium in the lumen were also observed. Also Farhad Khali et al., (1992) postulated that Continued lead-feeding resulted in atrophy of tubular lining cells, tubular dilation and interstitial fibrosis also mitochondrial abnormalities were evident in the lead-treated animals. The mitochondria were more rounded than elongated.

By electron microscopic examination we found The cells lining the proximal tubule showed vacuolated mitochondria, their microvilli were few and the lumen was wide. These results are agreed with those of Deveci et al., (2011) who studied the ultrastructural effects of lead on the kidney cortex of rats, exposed to drinking water containing lead acetate for a period of 2 months. The ultrastructural alterations found were, increase in lysosomal structures and pinocytic vesicles as well as vacuolated mitochondria in proximal tubule cells. WHO, (1991) postulated that The proximal tubular cells are particularly vulnerable owing to their high energy demand such as reabsorptive and secretory functions. Also Kathuria et al.,(2004) stated that Effects of lead on renal systems are mediated through peroxidative destruction to membranes. Lead accumulates in mitochondria and causes both structural and functional changes. The effects include mitochondrial enlargement, prevention of respiratory functions and energy construction. so energy dependent processes including tubular transport are prevented

The administration of curcumin, vitamin C and lead acetate to rats showed that the proximal tubules, the distal tubules and the glomeruli appeared like normal, so administration of both curcumin with vitamin C showed the best results than given alone in lead acetate intoxication during pregnancy and lactation on development of kidney. This in agreement with Soliman et al., (2015) who reported that The kidney in curcumin and lead acetate administered rats showed renewal in normal nephron structure with minor congestion of renal blood vessel and intraluminal hyaline casts are less. Also Mervat et al., (2012) found that Curcumin treatment in lead acetate treated rats showed slight protective effect against histopathological changes in the kidney tissues. Ambali et al., (2011) reported the protective effect of vitamin C against lead induced renal damage. they showed that the pre treatment with 100 mg/kg of vitamin C ameliorated the toxicological effect of lead in rats. Also El-Neweshy and El-Sayed, (2011) postulated that Vitamin C-lintake in lead acetate treated rats, like the control group, showed normal renal construction and histology. Jabeen et al., (2011) reported that vitamin C provided protection to developing kidney by decreasing concentration of lead acetate in blood.
Fig. (1-A): A light photomicrograph of kidney section of control rat fetus aged 18 days prenatal showing: renal cortex show immature forms of renal developmental stages: Renal vesicle (V), comma shape (C), S-shape (S), glomerulus (G). Note (CD) collecting duct, proximal tubule (P) and distal tubule (d). (HX&E X 400)

Fig. (1-B): A light photomicrograph of kidney section of control rat fetus aged 18 days prenatal: glomerulus (G), proximal tubule (P), distal tubule (D). Note: the connective tissue is minimal in amount (yellow arrow) (Masson's trichrome x 400)
Fig. (1-C): An electron photomicrograph of ultrathin section in kidney of control rat fetus aged 18 days prenatal showing: A part of proximal convoluted tubule lined with columnar cells which have a large rounded nuclei (N), many mitochondria (m), apical microvilli (MV). Notice basal lamina (BL). (E.M X 1500)

Fig. (2-A): A light photomicrograph of kidney section of rat fetus aged 18 days prenatal treated with lead acetate showing: the proximal convoluted tubule are dilated (L) and the lining epithelium is flattened (green arrow). some tubules show discoumated cells (star) which accumulate in its lumen. Some distal tubules (D) are degenerated. Note the glomerulus (G) appears intact. (HX&E X 400)
Fig. (2-B): A light photomicrograph of kidney section of rat fetus aged 18 days prenatal treated with lead acetate showing: increased the connective tissue (yellow arrow) around the glomerulus (G) and the proximal tubule (P) and distal tubule (d). (Masson’s trichorome x 400)

Fig. (2-C): An electron photomicrograph of ultrathin section in kidney of rat fetus aged 18 days prenatal treated with lead acetate showing: A part of proximal convoluted tubule lined with cuboidal cells which have ruptured brush border (green arrow), destructed microvilli (MV) some mitochondria (m) are vacuolated. Notice basal lamina (BL), the lumen of the tubule (L). (E.M X 1500)
Fig. (3-A): A light photomicrograph of kidney section of rat fetus aged 18 days prenatal treated with lead acetate plus curcumin and vitamin C showing: The proximal convoluted tubule (P), the distal tubule (D) and the glomeruli (G) appear like normal. Note (CD) collecting duct. (HX & E X 400)

Fig. (3-B): A light photomicrograph of kidney section of rat fetus aged 18 days prenatal treated with lead acetate plus curcumin and vitamin C showing: Minimal amount of connective tissue (yellow arrow) between The glomeruli (G), proximal tubules (P) and distal tubule (D). Note the collecting duct (CD). (Masson's trichorome x 400)
Fig. (3-C): An electron photomicrograph of ultrathin section in kidney of rat fetus aged 18 days prenatal treated with lead acetate plus curcumin and vit C showing: A part of proximal convoluted tubule lined by cuboidal cell which has rounded nucleus (N), elongated mitochondria (m), apical microvilli (MV). Note basal lamina (BL).

(E.M X 1500)

Fig. (4-A): A light photomicrograph of kidney section of control newborn rat aged 2 weeks after birth showing: mature glomerulus (G) which has parietal layer (yellow arrow), renal space (S). The proximal tubule (P) consists of columnar with rounded basal vesicular nuclei (n). The distal convoluted tubules (D) have low cubical cells with vesicular nuclei and wide lumen.

(HX&E X 400)
Fig. (4-B): A light photomicrograph of kidney section of control newborn rat aged 2 weeks after birth showing: normal glomeruli (G), proximal tubule (P), distal tubule (D). (Masson's trichrome x 400).

Fig. (4-C): An electron photomicrograph of ultrathin section in kidney of control newborn rat aged 2 weeks after birth showing: A part of proximal convoluted tubule lined with columnar cells which have a large rounded nuclei (N) with prominent nucleolus (n), elongated mitochondria (m), (E.M X 1500) apical microvilli (MV). Notice the basal lamina (BL).
Fig. (5-A): A light photomicrograph of kidney section of newborn rat aged 2 weeks after birth treated with lead acetate showing: Destruction of the glomeruli (G) with wide renal space (s). The proximal (P) and the distal tubules (d) are degenerated. Notice the nearly intact collecting tubules (CD) and wide spaces in interstitial tissue (yellow arrows). (HX&E X 400)

Fig. (5-B): A light photomicrograph of kidney section of newborn rat aged 2 weeks after birth treated with lead acetate showing: Condensed connective tissue (blue arrow) around the glomerulus (G) and the proximal (P) and distal tubules (d).
(Masson's trichrome x 400)
Fig. (5-C): An electron photomicrograph of ultrathin section in kidney of newborn rat aged 2 weeks after birth treated with lead acetate showing: Apart of proximal convoluted tubule lined with columnar cells which have oval nucleus (N). Its cytoplasm

Fig. (6-A): A light photomicrograph of kidney section of newborn rat aged 2 weeks after birth treated with lead acetate plus curcumin and vitamin C showing: Intact glomeruli (G) with renal space (s), proximal (P) and distal tubule (d). Notice some proximal tubules show few vacuolations (HX&E X 400)
Fig. (6-B): A light photomicrograph of kidney section of newborn rat aged 2 weeks after birth treated with lead acetate plus curcumin and vitamin C showing: Minimal amount of connective tissue in the interstitial space around glomerulus (G), proximal tubule (P) and distal tubule (d).

(Masson's trichrome x 400)

Fig. (6-C): An electron photomicrograph of ultrathin section in kidney of newborn rat aged 2 weeks after birth treated with lead acetate plus curcumin and vit C showing: A part of proximal convoluted tubule lined with columnar cell which has large oval nucleus (N), elongated mitochondria (m), less vacuoles (v). Notice microvilli (MV) and basal lamina (BL).

(EM X 1500)
References:


**Fig. (1) A:** Renal vesicle (V), comma shape (C), S- shape (S), glomerulus (G). (CD) collecting duct, proximal tubule (P) and distal tubule (d). (H&E X 400). **B:** glomerulus (G), proximal tubule (P), distal tubule (D). connective tissue minimal in amount (arrow) (Masson’s trichrome x 400). **C:** A part of proximal convoluted tubule lined with columnar cells have a large rounded nuclei (N), mitochondria (m), microvilli (MV). basal lamina (BL). (E.M X 1500)

**Fig. (2) A:** Proximal tubule dilated (L) epithelium flattened (arrow). discoumated cells (star). distal tubules (D) degenerated. (H&E X 400). **B:** increased connective tissue (arrow) glomerulus (G) proximal tubule (P) and distal tubule (d). (Masson’s trichrome x 400) **C:** ruptured brush border (arrow), destructed microvilli (MV) mitochondria (m) basal lamina (BL). (E.M X 1500)

**Fig. (3) A:** The proximal tubule (P), the distal tubule (D) and the glomerulus (G). (CD) collecting duct. (H&E X 400). **B:** Minimal connective tissue (arrow) between The glomeruli (G), proximal tubules (P) and distal tubule (D). collecting duct (CD). (Masson’s trichrome x 400). **C:** rounded nucleus (N), elongated mitochondria (m), apical microvilli (MV). basal lamina (BL). (E.M X 1500)

**Fig. (4) A:** Mature glomerulus (G) parietal layer (arrow), renal space (S). The proximal tubule (P) consists of columnar with rounded basal vesicular nuclei (n). The distal tubules (D) (H&E X 400). **B:** glomeruli (G), proximal tubule (P), distal tubule (D). (Masson’s trichrome x 400). **C:** A part of proximal tubule lined with columnar cells large rounded nuclei (N) prominent nucleolus (n), elongated mitochondria (m), apical microvilli (MV). the basal lamina (BL). (E.M X 1500)

**Fig. (5) A:** Destruction of the glomeruli (G) wide renal space (s). The proximal (P) and the distal tubules (d) are degenerated. collecting tubules (CD) and wide spaces in interstitial tissue (yellow arrows). (H&E X 400). **B:** connective tissue (arrow) around the glomerulus (G) and the proximal (P) and distal tubules (d) (Masson’s trichrome x 400). **C:** proximal tubule with oval nucleus (N), cytoplasm show extension (C) and has mitochondria (m) and apical vacuoles (V). the microvilli (MV) and basal lamina (BL). (E.M X 1500).

**Fig. (6) A:** A light photomicrograph of kidney section of rat fetus aged 2 weeks after birth treated with lead acetate plus curcumin and vitamin C showing: Intact glomeruli (G) with renal space (s), proximal (P) and distal tubule (d). Notice some proximal tubules show few vacuolations (H&E X 400). **B:** Minimal amount of connective tissue in the interstitial space around glomerulus (G),
proximal tubule (P) and distal tubule (d). (Masson's trichrome x 400). (C)= An electron photomicrograph of ultrathin section in kidney of rat fetus aged 2 weeks after birth treated with lead acetate plus curcumin and vit C showing: A part of proximal convoluted tubule lined with columnar cell which has large oval nucleus (N), elongated mitochondria (m), less vacuoles (v). Notice microvilli (MV) and basal lamina (BL). (E.M X 1500).

Fig.(4): An electron photomicrograph of ultrathin section in kidney of rat fetus aged 18 days showing: A= (control group) showing: A part of proximal convoluted tubule lined with columnar cells which have a large rounded nuclei (N), many mitochondria (m), apical microvilli (MV). Notice basal lamina (BL). (E.M X 1500). B= (treated group with lead acetate) showing: A part of proximal convoluted tubule lined with cuboidal cells which have ruptured brush border (green arrow), destructed microvilli (MV) some mitochondria (m) are vacuolated. Notice basal lamina (BL), the lumen of the tubule (L). (E.M X 1500). C= (treated with lead acetate plus curcumin and vit C): showing A part of proximal convoluted tubule lined by cuboidal cell which has rounded nucleus (N), elongated mitochondria (m), apical microvilli (MV). Note basal lamina (BL). (E.M X 1500).

Fig.(5): A light photomicrograph of kidney section of control rat fetus aged 2 weeks after birth showing: A=: mature glomerulus (G) which has parietal layer (yellow arrow), renal space (S). The proximal tubule (P) consists of columnar with rounded basal vesicular nuclei (n). The distal convoluted tubules (D) have low cubical cells with vesicular nuclei and wide lumen. (HX&E X 400). B=: normal glomeruli (G), proximal tubule (P), distal tubule (D). (Masson’s trichrome x 400).

Fig. (6): A light photomicrograph of kidney section of rat fetus aged 2 weeks after birth treated with lead acetate showing: Destruction of the glomeruli (G) with wide renal space (S). The proximal (P) and the distal tubules (d) are degenerated. Notice the nearly intact collecting tubules (CD) and wide spaces in interstitial tissue (yellow arrows). (HX&E X 400)