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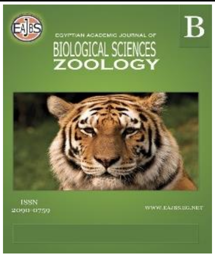

Modulatory Role of Zeolite against Cadmium Chloride-Induced Renal Damage In Pregnant Rats and Their Fetuses

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ABSTRACT

Cadmium (Cd) is a heavy metal and a significant inorganic toxin that is commonly found throughout the environment. The human body, as well as the kidneys, tend to accumulate Cd. Research conducted on both humans and animals suggests that exposure to cadmium can lead to significant skeletal damage, specifically osteoporosis. Based on the prevalence of cadmium in the environment and its frequent use, we studied the effect of its toxicity on pregnant rat and their fetuses and the effect of zeolite on them. The experimental rats were divided into 5 groups (n=8, each). The 1st group received distilled water as a normal control group. The 2nd received tween^{80} and served as tween^{80} group. The 3rd was administrated with zeolite dissolved in tween^{80}then in distilled water at a dose (100mg\kg\day). The 4th was administrated with cadmium chloride at dose (30mg\kg\day). The 5th was administrated with a combination of both zeolite and cadmium chloride. All groups were administered the materials orally from the 6th day to the 15th day of gestation. All animals were sacrificed at the end of gestation periods (on the 20th day). Our obtained results showed adverse histological alterations in maternal and fetal kidneys. Also, $CdCl_2$ (cadmium chloride) induced remarkable changes in the levels of kidney functions. On the other hand, results showed incomplete ossification of the skull, metacarpals, and phalanges of the toes of fetuses' skeletons. Zeolite administration markedly ameliorated the altered renal histological and physiological changes as well as the skeletal deformity in fetuses induced by cadmium.

INTRODUCTION

According to the industrial revolution that started in the 19th century, there is an increase in heavy metals usage in today's industry. Heavy metals can easily enter the food chain because of their high resistance to environmental conditions. Due to the long biological half-life, slow metabolism, and low elimination rate of heavy metals, they can accumulate in the placenta, small intestine, liver and kidney, and cause toxic effects (Şensoy, 2023).

Cadmium, a heavy metal, is found in various locations and is highly toxic even at minimal levels of exposure. It has both immediate and long-term impacts on both human health and the environment. Widely Cd is used in industrial applications according to its unique chemical and physical properties (Krichah *et al*., 2003). The main routes of exposure to cadmium include inhalation of fumes and dust containing cadmium, accidental ingestion through contaminated hands and food, and passive/active smoking (Ali *et al.*, 2023).

Cadmium builds up in the body's soft tissues and remains in the system for a long time, with a half-life of approximately 15-30 years in humans, mainly due to its slow elimination rate from the body (Henson and Chedrese, 2004).

Exposure to cadmium has been demonstrated to lead to the development of cancer and genetic mutations. (Waalkes *et al*., 2003; Huff *et al*., 2007). Cd has also been associated with a variety of harmful effects on reproductive organs. (Burukoglu and Bayçu, 2008; Aprioku *et al*., 2009). Cd has been demonstrated to disrupt ovarian functions, leading to ovulation failure and hindering implantation (Zhang *et al*., 2008; Obianime *et al*., 2011).

Furthermore, exposure to cadmium was found to cause skeletal demineralization by directly interacting with bone cells, reducing mineralization and inhibiting the formation of collagen and procollagen C-proteinases (Rahimzadeh *et al*., 2017).

The main targeted organ for cadmium accumulation is the kidney (Zhang *et al.*, 2024*)*. Chronic exposure to Cd causes severe nephrotoxicity in humans and animals. Renal dysfunction induced by cadmium may be due to proximal tubular damage affecting the passive paracellular pathway and a decrease in active transcellular ion transport (Jacquillet *et al.*, 2007). Based on current understanding, renal tubular damage is likely the most significant health impact, involving various mechanisms such as essential element replacement, inflammatory response, autophagy, apoptosis, and epigenetic alterations (Järup *et al*., 1998, Zhang *et al.*, 2024).

Zeolites are hydrogenated aluminium silicate minerals (Barbosa *et al*., 2016). They consist of interconnected tetrahedrons of aluminium oxide (AlO_4) and silicon dioxide (SiO_4) (Moshoeshoe *et al*., 2017). There are about 40 naturally occurring zeolites; the most commonly degraded form is clinoptilolite. Natural zeolites are formed in both volcanic and sedimentary rocks, according to the US Geological Survey (Bahgaat *et al*., 2020). On the other hand, approximately 150 synthetic zeolites have been created for particular uses, with the most famous being zeolite A, zeolites X, and Y. (Bahgaat *et al*., 2020).

Zeolites possess ion exchange and adsorption properties that can enhance the utilization of feed nitrogen in animal nutrition, decrease the occurrence of intestinal illnesses in young pigs and ruminants, manage the moisture and ammonia levels of animal manure, purify water in aquaculture circulatory incubators, supply fish farming with oxygen-enriched air, and minimize the nitrogen level of feed and brood run-off waters (Papaioannou *et al*., 2005; El-Nile *et al*., 2021).

According to the properties of zeolites, they are used in large quantities in a variety of commercial processes. The biggest application of zeolites is the purification of water. Other applications include catalysis, adsorbents and many applications of natural zeolites, including soil improvers and nutrient release agents in horticulture (Weckhuysen and Yu, 2015).

Zeolites have the ability to act as host matrices for the containment and stabilization of metal clusters or nanoparticles, creating versatile composites with excellent properties. Thanks to these unrivaled properties, due to their high hydrothermal stability and cost-effectiveness. Zeolites are extensively utilized as efficient catalysts, detergents, adsorbents, and ion exchangers in various chemical processes (Davis, 2002; Mintova *et al*., 2015).

Zeolites are interested in agriculture due to their main properties: high cation exchange, high adsorption capacity, and high water-holding capacity (Hedström, 2001). They improve soil properties by raising soil moisture and preventing hydraulic conductivity; also zeolites were used with fertilizers to help buffer soil pH (Cataldo *et al*., 2021).

Natural zeolites have beneficial effects on the body’s detoxification, mineral metabolism, immune system, blood circulation, and digestion (Hecht 2010). According to a study carried out by Pavelić *et al*. (2001), natural clinoptilolite zeolites act as anticancer therapeutic agents in vivo animal study and tissue culture cells. They are administrated orally in mice and dogs to improve all over health state, elongation of life span, and decrease tumor size (Moshoeshoe *et al*., 2017).

Synthetic zeolites have numerous benefits compared to natural ones. They are more efficient in radioactive waste removal from the environment (Lonin *et al*., 2015; Abdel Moamen *et al*., 2015), also they show a much higher capacity of adsorption for heavy metals like Cd^{2+}, Pb^{2+}, Zn^{2+}, etc. (He *et al*., 2016; Kozera-Sucharda *et al*., 2020). Accordingly, this study aims to evaluate the possible ameliorative role of zeolites against the hazardous effects of CdCl2 on the kidneys of pregnant rats and their pups.

MATERIALS AND METHODS

1-Experimental Animals:

The Albino rat (*Rattus norvegicus*) is an excellent choice for conducting experimental teratological studies due to its many advantages. It has a short gestation period of approximately 21 days. Additionally, rats are known for their genetic stability and exhibit a very low rate of spontaneous malformation (Kohn and Clifford, 2007; Modlinska and Pisula, 2020). Experimental animals (40) adult female albino rats, aged 12 weeks and weighing 160-190 g. They were mated with males; the vaginal smears were examined under light microscope. At the estrus phase in females, the existence of sperms in vaginal smears indicates the zero day of gestation.

2- Treatment and Doses:

Zeolite in a dose of 100 mg/kg/day was dissolved in tween eighty and then in distilled water (Saribeyoglu *et al*., 2011). Cadmium chloride at a dose of 30 mg/kg/day was dissolved in distilled water (Oluranti *et al*., 2021).

3. Experimental Design:

After mating, the pregnant rats were divided into 5 groups (n=8) according to the received substance as follows:

Group 1: 8 rats received distilled water (normal control group) from the 6^{th} day to the 15^{th} of gestation.

Group 2: 8 rats received $tween^{80}$ (control $tween^{80}$ group) from the 6^{th} day to the 15^{th} of gestation.

Group 3: 8 rats received zeolite (dissolved in 1% $tween^{80}$) in a dose of 100 mg/kg per day from the 6^{th} day to the 15^{th} of gestation.

Group 4: 8 rats received Cadmium Chloride (dissolved in distilled water) in a dose of 30 mg/kg/day from the 6^{th} day to the 15^{th} day of gestation.

Group 5: 8 rats received zeolite concurrently with $CdCl_2$ from the 6^{th} day to the 15^{th} day of gestation by the same doses as in groups 3&4.

Animals of all groups were sacrificed at the end of gestation periods (on the 20^{th} day of gestation). Maternal kidneys and whole fetuses were quickly removed and collected to be used in our study.

4-Histological Examinations:

The maternal and fetal kidneys were extirpated**,** fixed in 10% formalin, dehydrated in ascending grades of ethyl alcohol, cleared in xylol, embedded in molten paraplast at 56°C, and cut at 5µ on rotator microtome. The paraffin sections were stained with haematoxylin and eosin (Drury and Wallington, 1980). Histopathological studies were conducted using light microscopy and photomicrographs were captured.

5-Biochemical Investigation:

Maternal blood samples were collected and centrifuged at 10000 xg for 10 minutes at 4°C. The resulting supernatant is serum. the serum should be stored and transported at –20°C or lower.

Kidney function tests were measured to determine serum creatinine and urea levels by using a 96-well plate according to the method described by (REF).

6-Skeletal Staining And Examination:

1-Fetuses were carefully eviscerated.

2-They were fixed in 95% ethyl alcohol for hardening of the specimens.

3-Staining with double staining of fetal skeletons for cartilage (blue) and bone (red) according to the method described by Peters (1977).

4-Fetuses were stained with alcian blue solution at first then treated with a relatively weak solution of potassium hydroxide which renders the muscles transparent then stained with Alizarin red solution.

5.After staining, the specimens were kept in glycerin. The skeleton was examined under the dissecting binocular microscope to study any malformation and shortening in the bones of fetuses.

7-Statistical Analysis:

Results have been analyzed by the prism program version (5). Comparison between more than two different groups was carried out using the one-way analysis of variance (ANOVA) followed by Tukey-Kramer's Multiple Comparison Test **(Armitage and Berry, 1987)**, where $P<0.05$ was considered significant**.** All the values were presented as means ± standard errors of the means (S.E.M.)**.**

RESULTS

1-Histological Examination:

1.1-The Effect Of Zeolite And Cadmium Chloride On The Maternal Kidney:

Control Groups:

The renal cortex was packed with renal (Malpighian) corpuscles, glomerular capillaries, Bowman's capsules, and urine space. Proximal convoluted tubules have a tiny lumen surrounded by cubical cells with round nuclei at the base of the tubule. The lumen of the distal convoluted tubules was large and bordered by simple cubical cells with sphere nuclei in the middle or at the apex of the tubule walls (Fig. 1 A and B)

Zeolite Group:

The most of renal glomeruli and tubules appeared normal. There was no lymphatic intrusion. The glomeruli showed normal and standard size inside Bowman's capsule (Fig. 1 C, D and E).

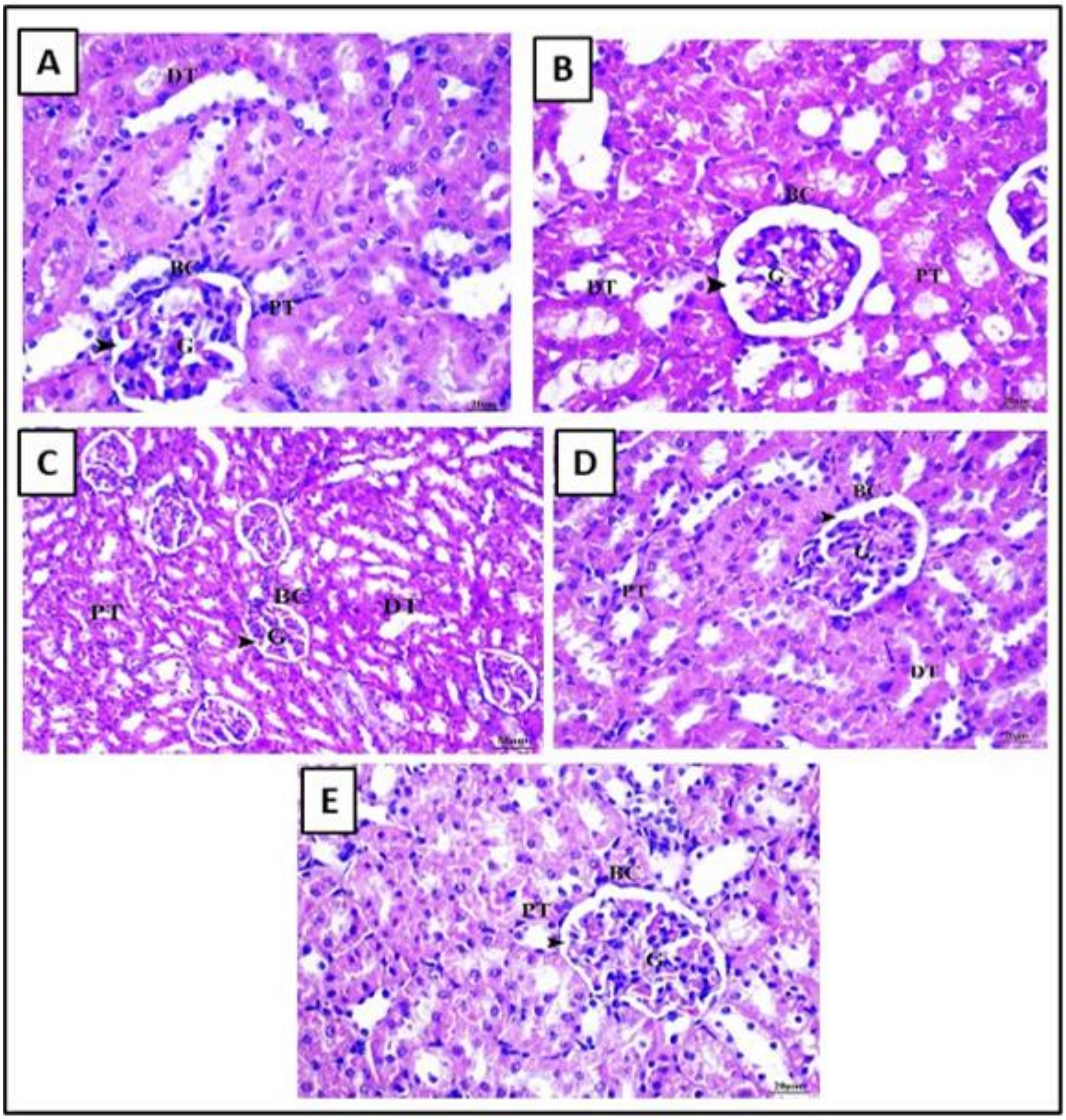

Fig. 1: photomicrographs of histology of kidney of pregnant rats: (A) normal control group, (B) control tween[80], (C), (D), (E) zeolite group showed normal histological structure of Bowman's capsule (BC), glomeruli (G), proximal tubule (PT), distal tubule (DT), capsular space (Head arrow) congestion (Asterisk) lymphatic.

Cadmium Chloride Group:

It showed a glomerular disparity in size and shape, with some glomeruli contracting in size because of atrophied glomerular tuft and cystic appearance. Some glomeruli were seen to be vacuolated, fractured, and compacted. In the proximal and distal convoluted tubules, cell nuclei exhibited complete or partial damage, implying dissolution. Additionally, the nucleus has been observed to compact (pyknosis). Hydropic degeneration resulted in blurry development of the convoluted tubule endothelial lining cell. There was inflammatory cell infiltration in the injured tubules and renal corpuscles. Renal tubules were shown to have disrupted epithelial linings and desquamation. Within tubular cells, cellular remains were found (lumen cast) **(**Fig. 2**)**.

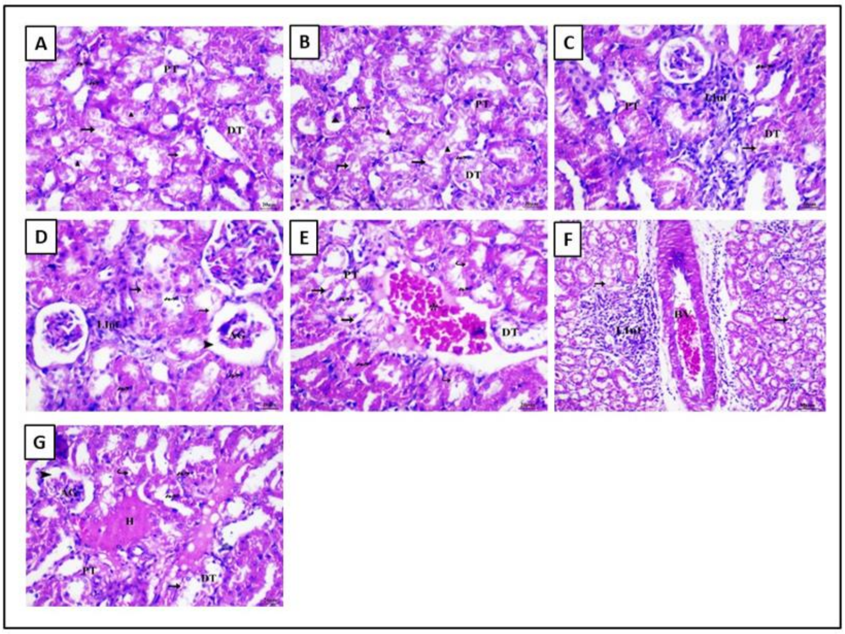

Fig. 2: photomicrographs of histology of kidney of pregnant rats in cadmium chloride group: showed that some glomeruli (G) contracting in size and some were vacuolated, fractured, and compacted. atrophied glomeruli (AG), In proximal tubule (PT) and distal tubule (DT), cell nuclei exhibited complete or partial damage, hemorrhage (H), capsular space (Head arrow), congestion (Asterisk) lymphatic infiltration (LInf), degenerated renal tubule (Arrow), pyknotic nuclei (Zigzag arrow), luminal cast (Triangle) and cytoplasmic vacuoles (Curved arrow).

Zeolite and Cadmium Chloride Group (Zeo+$CdCl_2$) Group:

Some tubular epithelial cells in renal convoluted tubules exhibited moderate vacuolation and degenerative changes. The glomerulus is of normal size within Bowman's capsules with a normal volume of capsular space. Numerous glomeruli were atrophied. Few pyknotic nuclei are seen in only a few tubules and degeneration of the tubular hydropic membrane. There was no evidence of lymphatic invasion. There was no area of hemorrhage. Congestion was noted to be mild **(**Fig. 3**)**.

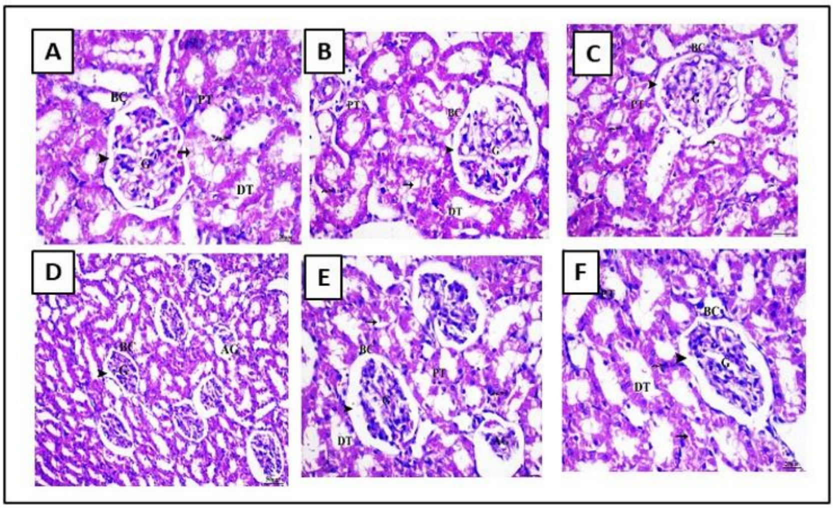

Fig. 3: photomicrographs of histology of the kidney of pregnant rats in Zeo+$CdCl_2$ group: showed normal size of glomeruli (G), numerous atrophied glomeruli (AG), Bowman's capsules (BC) with normal volume of capsular space (Head arrow), moderate degenerated renal tubule (Arrow) and few pyknotic nuclei (Zigzag arrow).

1.2- The Effects on Fetal Kidney:

Control Groups:

Bowman's capsule includes intact blood vessels glomerulus with clear capsular space. Renal tubules are normal and lined with simple epithelial cells and conspicuous spherical centrally located nuclei (Fig. 4 A and B).

Zeolite Group:

The examination showed standard renal tissue structure with the normal organization of Bowman's capsule, glomeruli, and renal tubules (Fig. 4 C and D).

Cadmium Chloride Group:

The fetal kidney showed obvious degeneration of the basement membrane enclosing the glomeruli and epithelial cells lining the renal tubules, as well as tubules that were destroyed. In the lining cells of the tubules, there was nuclei degradation, pyknotic nuclei, hazy swelling, and disintegration (Fig. 4 E and F).

Zeo+$CdCl_2$ Group:

The examination showed a certain degree of recovery and restored the normal architecture. Most of Bowman's capsule and glomeruli appeared with the usual form and size, but others revealed atrophied glomeruli. Many renal tubules appeared normal configuration however other tubules revealed little extent of degeneration (Fig. 4 G, H and I).

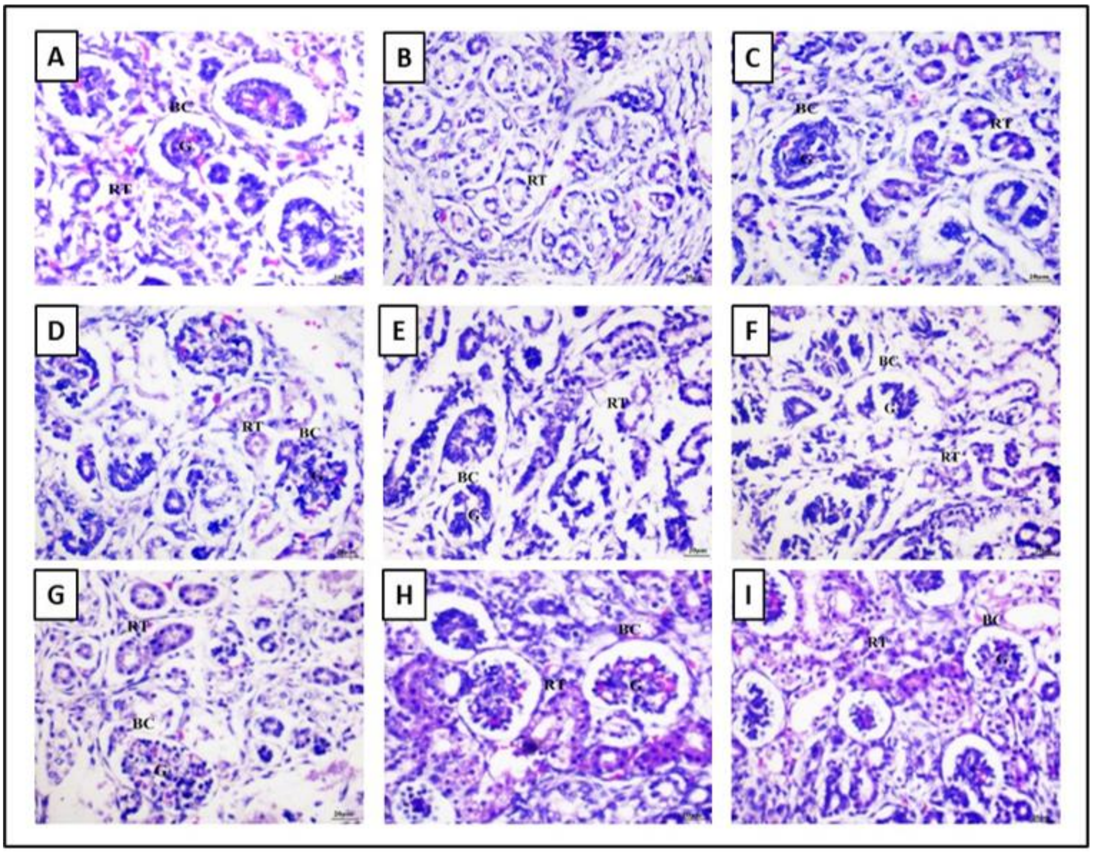

Fig. 4: photomicrographs of histology of fetal kidney: (A) normal control group, (B) controls tween[80] and (C), (D) zeolite group showed the normal structure of Glomeruli (G), renal tubule (RT), Bowman's capsule (BC). (E), (F) cadmium chloride group showed degeneration of glomeruli (G) membrane, destroyed tubules, pyknotic nuclei, hazy swelling, and disintegration. (G), (H), (I) Zeo+$CdCl_2$ group showed the usual form and size of Bowman's capsule (BC) and glomeruli (G), but others revealed atrophied glomeruli, little extent of degeneration of renal tubules (RT), however many tubules appeared normal configuration.

2-Biochemical Study:

Maternal Kidney Functions:

Serum creatinine levels in cadmium chloride and Zeo+CdCl2 were higher by 221.85% and 107.41% respectively than the normal control group. On the other hand, the levels in zeolite and Zeo+CdCl2 groups were lower by 71.23% and 35.58% respectively as compared to the cadmium chloride group (Fig. 5). Serum urea levels in cadmium chloride and Zeo+CdCl2 group increased significantly by 175.5% and 44.2% respectively comparing with the normal control group, while in zeolite and Zeo+CdCl2 decreased by 68.46 and 47.67% respectively comparing with CdCl2 (Fig. 6).

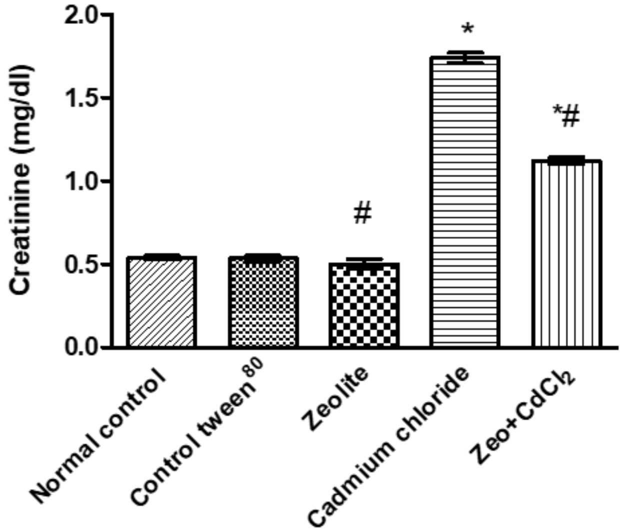

Figure 5: Effect on serum creatinine levels at 20th day of gestation: Each value indicates the mean ± S.E.M of 6 animals. Statistical analysis was carried out by One Way ANOVA followed by Tukey- Kramer Multiple Comparison Test ANOVA followed by Tukey-Kramer Multiple Comparison Test. *significantly different from the normal control group at P < 0.05. *significantly different from the cadmium chloride group at P < 0.05.

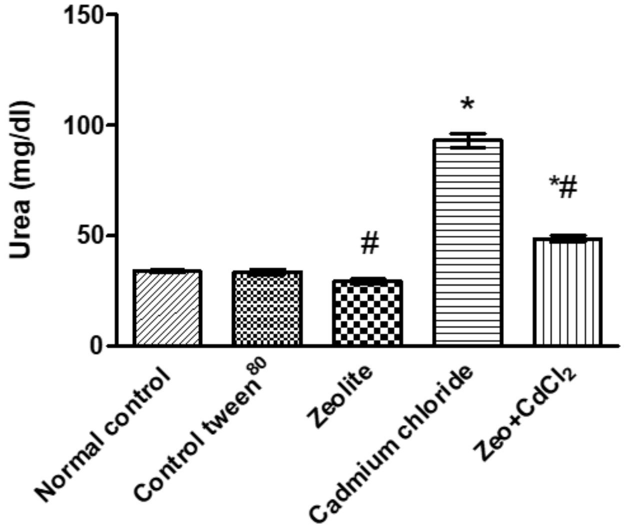

Fig. 6: Effect on level of serum urea levels at 20th day of gestation: Each value indicates the mean ± S.E.M of 6 animals. Statistical analysis was carried out by One Way ANOVA followed by Tukey- Kramer Multiple Comparison Test ANOVA followed by Tukey-Kramer Multiple Comparison Test. *significantly different from the normal control group at P < 0.05. #significantly different from the cadmium chloride group at P < 0.05.

3- Skeletal study:

On the 20th day of gestation, the cleared cartilage and bone preparations of control rat fetuses have been designated. The data showed well ossification of the skull (Fig.7 panel 1, A and B), ribs (Fig.7 panel 2, A and B), vertebrae, and hind limbs (Fig.7 panel 3, A and B).

The skeleton of fetuses administrated with zeolite (100mg/kg) showed normal ossified bones (Fig.7 panel 1, C, 2, C and 3, C).

In the fetuses treated with cadmium chloride (30mg/kg), skeleton showed lack ossification of skull (Fig.7 panel 1, D), metacarpals and phalanges of toes were completely non-ossified, thoracic vertebrae were non-ossified and also its vertebral ribs (Fig.7 panel 2, D), metatarsus and phalanges of the toes were completely non-ossified (Fig.7 panel 3, D). On the other hand, the skeleton of fetuses treated with Zeo+$CdCl_2$ showed mild ossification of skull (Fig.7 panel 1, E), metacarpals and phalanges of the toes were mild ossified, thoracic vertebrae were mild and also its vertebral ribs (Fig.7 panel 2, D), metatarsus and phalanges of the toes were completely weak ossified (Fig.7 panel 3, D).

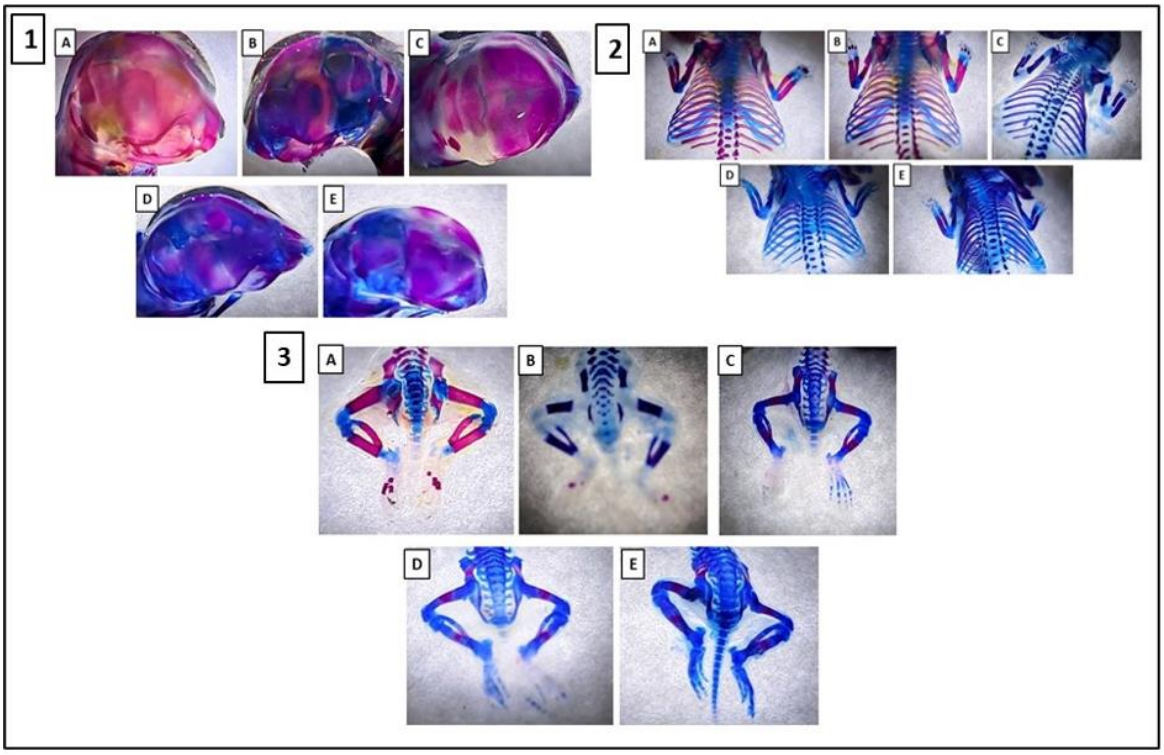

Fig. 7: Photomacrographs of the Skeleton of Fetuses on the 20th day of gestation: Panel 1 skull bones of (A) normal control group, (B) control tween80 group and (C) zeolite group showed well ossification. (D) $CdCl_2$ group showed a lack of ossification of the skull. (E) Zeo+$CdCl_2$ group showed mild ossification of the skull. Panel 2 vertebral column and ribs bones of (A) normal control group, (B) control tween80 group and (C) zeolite group showed complete ossification. (D) $CdCl_2$ group showed non-ossification vertebral ribs. (E) Zeo+CCl_2 group showed mild ossified vertebral ribs. Panel 3 hind limb and sacrum of (A) normal control group, (B) control tween80 group and (C) zeolite group showed complete ossification. (D) $CdCl_2$ group showed completely non-ossified metatarsus and phalanges of the toes. (E) Zeo+$CdCl_2$ group showed completely weak ossified metatarsus and phalanges of the toes.

DISCUSSION

The organs in charge of drug and xenobiotic metabolism are the kidney and liver. The liver and kidneys are first affected by chemical poisoning, then the other organs. Abdel-Daim *et al*. (2013) stated that the liver and kidney are chemically poisoned first, then the other organs. According to a study by Parzyck, *et al*., (1978), Cd exposure during pregnancy leads to several effects on kidney, blood pressure and essential elements rotation in the body, and also is the reason for other pathologies in the mothers and fetuses (Estrada *et al*., 2017).

In the present work and due to the administration of cadmium, maternal and fetal kidneys showed a glomerular disparity in size and shape, with some glomeruli contracting in size. Some glomeruli were seen to be vacuolated, fractured, and compacted. In renal tubules, cell nuclei exhibited complete or partial damage, implying dissolution. There was nuclei degradation, pyknotic nuclei, hazy swelling, and disintegration. There was inflammatory cell infiltration in the injured tubules and renal corpuscles. Renal tubules were shown to have disrupted epithelial linings and desquamation. Within tubular cells, cellular remains were found (lumen cast). That was found in previous studies by Brzóska *et al*. (2003) and Hamidian *et al*. (2020) which showed degeneration and hypertrophy of epithelial cells and dilation in glomeruli, pycnotic nuclei with focus necroses in urinary tubules and congestion of blood vessels.

Serum Creatinine and urea levels showed a significant increase in the dams that received $CdCl_2$ as compared to the control group. Such findings agree with the previous reports (Karami *et al*., 2022; Badawy *et al*., 2023) which observed an increase in creatinine and urea levels at a dose (3 mg/kg) of $CdCl_2$ administration in male Wister rats, also serum Creatinine and urea levels increased significantly compared to control groups at a dose (2 mg/kg) $CdCl_2$ in male SD rats (Huang *et al*., 2022).

The skeletal examination of $CdCl_2$ maternally treated fetuses observed a lack of ossification of the skull, metacarpals and phalanges of toes showed no signs of ossification, thoracic vertebrae were non-ossified, also its vertebral ribs. This was compatible with previous studies that showed Cd treatment during organogenesis leads to incomplete ossification of the skull, ribs and vertebrae, shortness of rib in some fetuses and reduced metacarpus ossification and cleft palate. (Salvatori *et al* (2004) and El-Sayed (2013) found that Cd treatment reduced metacarpus ossification and cleft palate. The most remarkable effects seen in the fetuses' skeleton of the Cd maternally treated group were delayed ossification of the head and vertebrae, as well as fused ribs and vertebrae (Argüelles, *et al*., 2013). The highest level of cadmium toxicity was found in the length of skeletons in the tibia, while the lowest was observed in the arm. In terms of the width of skeletons, the most toxic impact of cadmium was on the large diameter of the pelvis, with the lowest effect seen on the large diameter of the chest (Abdollahi, *et al*., 1998).

On the other hand, zeolite administration showed the normal histological structure of maternal and fetal kidneys. Most renal glomeruli and tubules appeared normal. There was no lymphatic intrusion. The glomeruli with normal and standard size inside Bowman's capsule. That was observed in the previous study by Sumer *et al.* (2022) Which found that kidneys of the zeolite group showed normal glomerulus and renal capsule and no pathological alterations in renal tubules.

Serum creatinine and urea levels recorded normal values in the zeolite group, as well as their levels decreased when the dams treated with zeolite with cadmium chloride which agreed with pablack and Zentek (2018) who found no effect on blood urea and creatinine concentration in dietary supplementation of zeolite.

Maternally treated fetuses with zeolite showed normal ossified bones. Zeo+$CdCl_2$ maternally treated showed mild ossification of skull, metacarpals and phalanges of toes, vertebrae, and ribs. According to Zarrintaj *et al*. (2020), Zeolites are used in bone tissue engineering due to their large surface area with no cytotoxicity, they assist in the process of grafts and coating synthesis because the implants are suitable as the primary framework to protect the cells from defects and improve cell proliferation and growth. Treatment with PMA-zeolite-clinoptilolite has beneficial effects on osteoporotic patients. The mechanisms by which it affects bone mineralization are still unclear (Pavelić *et al*., 2021).

Conclusion:

Our results showed that zeolite administration decreases the toxic effect of cadmium chloride on dams and their fetuses. This was demonstrated by a decrease in the side effects of cadmium on the histology and biochemistry of maternal and fetal kidneys, as well as improved skeleton ossification.

Declarations:

Ethical Approval: Experimental procedures were implemented following the ethical guidelines for research involving animals, and were approved by the standard guidelines of EDA (Approval number: NODCAR/1/16/2021).

Conflicts of Interest: There is no conflict of interest.

Informed consent: All the authors of this manuscript accepted that the article is submitted for publication in the Egyptian Academic Journal of Biological Sciences, B. Zoology, and this article has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

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REFERENCES

Abdel Moamen, O.A.; Ismail, I.M.; Abdelmonem, N. and Abdel Rahmana, R.O. (2015): Factorial design analysis for optimizing the removal of cesium and strontium ions on synthetic nano-sized zeolite. *Journal of the Taiwan Institute of Chemical Engineers*, 55:133–144.

Abdel-Daim, M.M.; Abuzead, S.M. and Halawa, S.M. (2013): Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. *PloS One,* 8(9): 72991.

Abdollahi, M.; Chafjiri, M.S. and Shariatpanahi, N.O.M. (1998): Skeletal and external teratogenic effects of cadmium during gestation in rats. *Acta Medica Iranica,* 36.

Ali, W.; Bian, Y.; Zhang, H.; Qazi, I. H.; Zou, H.; Zhu, J. and Liu, Z. (2023): Effect of cadmium exposure during and after pregnancy of female. *Environmental Pollutants and Bioavailability*, 35(1): 1- 8.

Aprioku, J. S.; Obianime, A. W. and Didia, B. C. (2009): Ocimum gratissimum Linn., cadmium and testicular structure of the male guinea-pig. *Journal of Experimental and Clinical Anatomy,* 8:8-12.

Argüelles-Velázquez, N.; Alvarez-González, I.; Madrigal-Bujaidar, E. and Chamorro-Cevallos, G. (2013): Amelioration of Cadmium-Produced Teratogenicity and Genotoxicity in Mice Given Arthrospira maxima (Spirulina) Treatment. Evidence-Based Complementary and Alternative Medicine, 2013:8.

Armitage, P. and Berry, G. (1987): Comparison of several groups. In: Statical Methods in Medical Research. Oxford: *Blackwell scientific publication,* pp. 186–213.

Badawy, S.; Abd-Elraouf, M.; Gab-Allah, M.S.A.; El-mashed, A.I.; Tantawy, A.A. and Amin, A.A. (2023): Nephroprotective Properties of Palm Dates and Olive Leaves Extracts on Cadmium-Induced Acute Renal Toxicity in Albino Rats. J*ournal of Advanced Veterinary Research,* 13(8): 1497-1506.

Bahgaat, A.K.; Hassan, H.E.; Melegy, A.A.; Abd-El kareem, A.M. and Mohamed, M.H. (2020): Synthesis and characterization of zeolite-Y from natural clay of Wadi

Hagul, Egypt. *Egyptian Journal of Chemistry,* 63:3791-3800.

Barbosa, G.P.; Debone, H.S.; Severino, P.; Souto, E.B.; da Silva, C.F. (2016): Design and characterization of chitosan/zeolite composite films — Effect of zeolite type and zeolite dose on the film properties. *Materials Science and Engineering: C,* 60:246-254.

Brzóska, M.M.; Moniuszko-Jakoniuk, J.; Piłat-Marcinkiewicz, B. and Sawicki, B. (2003): Liver and kidney function and histology in rats exposed to cadmium and ethanol. *Alcohol and Alcoholism,* 38(1): 2–10.

Burukoglu, D. and Bayçu, C. (2008): Protective effects of zinc on testes of cadmium-treated rats. *Bulletin of Environmental Contamination and Toxicology,* 81:521-524.

Cataldo, E.; Salvi, L.;Paoli, F.; Fucile, M.;Masciandaro, G.; Manzi, D.;Masini, C.M. and Mattii, G.B. (2021): Application of Zeolites in Agriculture and Other Potential Uses: A Review. *Agronomy,* 11(8).

Davis, M.E. (2002): Ordered porous materials for emerging applications. *Nature,* 417:813–821.

Drury, R.A.B. and Wallington, E.A. (1980): Carleton's Histological Technique. 5th Edition, Oxford University Press, New York.

El-Nile, A.; Elazab, M.; El-Zaiat, H.; El-Azrak, K.E.; Elkomy, A.; Sallam, S. and Soltan, Y. (2021): *In Vitro* and *In Vivo* Assessment of Diatery Supplimentation of Both Natural and or Nano-Zeolite in Goat Diets: Effectes on Ruminal Fermentation and Nutrients Digestibility. *Animals (Basal),* 11(8):2215.

El-sayed, A. (2013): Protective effect of zinc against cadmium toxicity on pregnant rats and their fetuses at morphological, physiological and molecular level. *African Journal of Biotechnology,* 12(16):2110-2119.

Estrada, T.J.; Sánchez, M.S.; Thévenod, F. and Barbier, O. (2017): Cadmium Handling, Toxicity and Molecular Targets Involved during Pregnancy: Lessons from Experimental Models. *International Journal of Molecular Sciences,* 18(7):1590.

Hamidian, G.; Mirdar, S.; Raee, P.; Asghari, K. and Jarrahi, M. (2020): Silymarin protects the structure of kidney in the neonatal rats exposed to maternal cadmium toxicity: A stereological study. *Veterinary Research Forum,* 11(2):143–152.

He, K.; Chen, Y.; Tang, Z. and Hu, Y. (2016): Removal of heavy metal ions from aqueous solution by zeolite synthesized from fly ash. *Environmental Science and Pollution Research,* 23(3): 2778–2788.

Hecht, K. (2010): Clinoptilolite-zeolite and montmorillonite minerals rich in SiO2: What are they? What can they achieve? Why are they so important for human health? Application of natural zeolites in medicine and cosmetology. ZEOMEDCOS. SWB, Baku-London, 7-45.

Hedström, A. (2001): Ion exchange of ammonium in zeolites: A literature review. *Journal of Environmental Engineering,* 127(8): 673–681.

Henson, M. C. and Chedrese, P. J. (2004): Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. *Experimental Biology and Medicine,* 229(5):383-92.

Huang, R.; Ding, L.; Ye, Y.; Wang, K.; Yu, W.; Yan, B.; Liu, Z. and Wang, J. (2022): Protective effect of quercetin on cadmium-induced renal apoptosis through cyt-c/caspase-9/caspase-3 signaling pathway. *Frontiers in Pharmacology,* 13:990993.

Huff, J.; Lunn, R. M.; Waalkes, M. P.; Tomatis, L. and Infante, P. F. (2007): Cadmiuminduced cancers in animals and in humans. *International Journal of Occupational and Environmental Health,* 13(2):202-212.

Jacquillet, G.; Barbier, O.; Rubera, I.; Tauc, M.; Borderie, A.; Namorado, M. C.; Martin, D.; Sierra, G. and Reyes, J. L. (2007): Cadmium causes delayed effects on renal function in the offspring of cadmium-contaminated pregnant female rats. *American*

Journal of Physiology-Renal Physiology, 293(5): 1450-1460.

Järup, L.; Berglund, M.; Elinder, C.G.; Nordberg, G. and Vahter, M. (1998): Health effects of cadmium exposure - a review of the literature and risk estimate. *Scandinavian Journal of Work Environment & Health,* 24 (1): 1-51.

Karami, E.; Goodarzi, Z.; Al Ghanbari; Bandegi, A.R.; Yosefi, Y. and Dehdashti, A. (2022): In vivo antioxidant and kidney protective potential of Atorvastatin against cadmium chloride-induced kidney injury in male Wistar rat. *Environmental Toxicology and Health,* 15(1): 1025-1036.

Kohn, D.F. and Clifford, C.B. (2007): Biology and Diseases of Rats. *Laboratory Animal Medicine*, 2002:121-165.

Kozera-Sucharda, B.; Gworek, B. and Kondzielski, I. (2020): The simultaneous removal of zinc and cadmium from multicomponent aqueous solutions by their sorption onto selected natural and synthetic zeolites. *Minerals, 10*: 343.

Krichah, R.; Ben Rhouma, K.; Hallègue, D.; Tébourbi, O.; Joulin, V.; Couton, D. and Sakly, M. (2003): Acute Cadmium Administration Induces Apoptosis in Rat Thymus and Testicle, but not Liver. *Polish Journal of Environmental Studies,* Vol. 12(5):589-594.

Lonin, A.Y.; Levenets, V.V.; Neklyudov, I.M. and Shchur, A.O. (2015): The usage of zeolites for dynamic sorption of cesium from wastewaters of nuclear power plants. *Journal of Radioanalytical and Nuclear Chemistry,* 303(1): 831–836.

Mintova, S.; Jaber, M. and Valtchev, V. (2015): Nanosized microporous crystals: emerging applications. *Chemical Society Reviews,* 44(20): 7207–7233.

Modlinska, K, and Pisula, W. (2020): The Natural History of Model Organisms: The Norway rat, from an obnoxious pest to a laboratory pet. *eLife*, 9, e50651.

Moshoeshoe, M.; Nadiye-Tabbiruka, M.S. and Obuseng, V. (2017): A Review of the Chemistry, Structure, Properties and Applications of Zeolites. *American Journal of Materials Science,* 7(5): 196-221.

Obianime, A. W.; Aprioku, J. S. and Ahiwe, N. J. (2011): Biochemical and hormonal effects of cadmium in female guinea pigs. *Journal of Toxicology and Environmental Health Sciences,* 3(2):39-43.

Oluranti, O.I.; Agboola, E.A.; Fubara, N.E.; Ajayi, M.O. and Michael, O.S. (2021): Cadmium exposure induces cardiac glucometabolic dysregulation and lipid accumulation independent of pyruvate dehydrogenase activity. *Annals of Medicine,* 53(1): 1108–1117.

Pablack, N. and Zentek, J. (2018): Effects of Dietary Arginine, Ornithine, and Zeolite Supplementation on Uremic Toxins in Cats. *Toxins (Basal),* 10(5):206.

Papaioannou, D.; Katsoulos, P.D.; Panousis, N.; and Karatzias, H. (2005): The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal disease: A review. *Microporous Mesoporous Mater,* 84(1): 161-170.

Parzyck, D.C.; Shaw, S.M.; Kessler, W.V.; Vetter, R.J.; Van Sickle, D.C. and Mayes, R.A. (1978): Fetal effects of cadmium in pregnant rats on normal and zinc deficient diets. *Bulletin of Environmental Contamination and Toxicology,* 19(2):206-14.

Pavelić, K.; Hadzija, M.; Bedrica, L.; Pavelić, J.; Dikić, I.; Katić, M.; Kralj, M.; Bosnar, M.H.; Kapitanović, S.; Poljak-Blazi, M.; Krizanac, S.; Stojković, R.; Jurin, M.; Subotić, B. and Colić, M. (2001): Natural zeolite clinoptilolite: new adjuvant in anticancer therapy. *Journal of Molecular Medicine*, 78: 708–720.

Pavelić, S.K.; Micek, V.; Bobinac, D.; Bazdulj, E.; Gianoncelli, A.; Krpan, D.; Žuvić, M.; Eisenwagen, S.; Stambrook, P.J. and Pavelić, K. (2021): Treatment of osteoporosis with a modified zeolite shows beneficial effects in an osteoporotic rat model and a human clinical trial. *Experimental Biology and Medicine*, 246(5): 529–537.

Peters, P.W.J. (1977): Double staining of fetal skeletons for cartilage and bone. In: Neubert D, Merker HJ, Kwasigroch TE: Methods in pre-natal toxicology. Stuttgart: Georg Thieme. P 153–154.

Rahimzadeh, M.R.; Rahimzadeh, M.R.; Kazemi, S. and Moghadamnia, A. (2017): Cadmium toxicity and treatment: An update. *Caspian Journal of Internal Medicine*, 8(3): 135–145.

Salvatori, F.; Talassi, C.B.; Salzgeber, S.A.; Spinosa, H.S. and Bernardi, M.M. (2004): Embryotoxic and long-term effects of cadmium exposure during embryogenesis in rats. *Neurotoxicology and Teratology,* 26 (5):673-680.

Saribeyoglu, K.; Aytac, E.; Pekmezci, S.; Saygili, S.; Uzun, H.; Ozbay, G.; Aydin, S. and Seymen, H.O. (2011): Effects of clinoptilolite treatment on oxidative stress after partial hepatectomy in rats. *Asian Journal of Surgery,* 34(4): 153-157.

Şensoy, E. (2023): Investigation of the effect of Cadmium chloride applied during pregnancy on the morphological parameters of mouse offspring and the protective role of melatonin. *Journal of Hazardous Materials Advances,* 9.

Sumer, E.; Hmitoglu, M.; Cumbul, A.; Ercan, S.; Bac, N. and Aydin, A. (2022): Determination of *In Vivo* efficacy and safety of zeolite as anew pleurodesis agent. *Toxicology Reports,* 9(2022): 1754-1765.

Waalkes, M. P., (2003): Cadmium carcinogenesis. Mutation Research, 533:107-120.

Weckhuysen, B.M. and Yu, J. (2015): Recent advances in zeolite chemistry and catalysis. *Chemical Socitety Reviews,* 44(20): 7022-7024.

Zarrintaj, P.; Mahmodi, G.; Manouchehri, S.; Mashhadzadeh, A.H.; Khodadadi, M.; Servatan, M.; Ganjali, M.R.; Azambre, B.; Kim, S.; Ramsey, J.D.; Habibzadeh, S.; Saeb, R.S. and Mozafari, M. (2020): Zeolite in tissue engineering: Opportunities and challenges. *MedComm,* 1(1): 5-34.

Zhang, K.; Long, M.; Dong, W.; Li, J.; Wang, X.; Liu, W.; Huang, Q.; Ping, W.; Zou, H.; Song, R.; Liu, G.; Ran, D. and Liu, Z. (2024): Cadmium induced kidney iron deficiency and chronic kidney injury by interfering with the iron metabolism in rats. *International Journal of Molecular Sciences*, 25(2).763.

Zhang, W.; Pang, F.; Huang, Y.; Yan, P. and Lin, W. (2008): Cadmium exerts toxic effects on ovarian steroid hormone release in rats. *Toxicology Letters,* 182:18-23.