Arq. Bras. Med. Vet. Zootec., v.76, n.4, e13194, 2024

http://dx.doi.org/10.1590/1678-4162-13194
Original Article - Veterinary Medicine

Zeolite abrogates cadmium-induced testicular damage in rats: implication of NF-κB/ TNF-α/IL-1β Pathway

[*Zeólito anula danos testiculares induzidos por cádmio em ratos: implicação da via NF-κB/ TNF-α/IL-1β*]

N.M. *Ibrahim*[1], A.M. *Kandil*[2], R.S. *Ali*[1], R. *Hesham*[1], S. *El-Ashram*[3,4], M.I. *Alquraishi*[5], R. *Abdel-Gaber*[6,*], M.A. *Dkhil*[1], A.A. *Elhemiely*[2]

[1]Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt
[2]Department of Pharmacology, Egyptian Drug Authority, EDA, Formerly NODCAR, Giza, Egypt
[3]College of Life Science and Engineering, Foshan University, Foshan 528231, Guangdong Province, China
[4]Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh, Egypt
[5]Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia
[6]Department of Zoology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia

ABSTRACT

Cadmium (Cd) is an environmental pollutant affecting various tissues and organs, including the testis. Many studies demonstrated that Cd toxicity causes testicular impairment through oxidative stress and inflammatory action. Therefore, this study aimed to demonstrate Cd's testicular toxicity and the protective action of zeolite against cadmium's deleterious effects. Adult male rats were given Cd at a dose of 30mg/kg/day for 28 consecutive days with or without zeolite, which was given at a dose of 100mg/kg/day for 28 days. Testis weight, sperm (count, motility, and abnormalities), serum testosterone and luteinizing hormone (LH), testicular enzymes Acid phosphatase (ACP) and Alkaline phosphatase(ALP), inflammatory cytokines Tumor Necrosis Factor Alpha (TNF-α) , interleukin-1beta (IL-1β) , and Nuclear Factor Kappa B (NF-κB) and oxidative stress were evaluated. Herein, we found that cadmium caused alterations in sperm characteristics, sex hormone disturbance, decline in testicular enzymes, elevated malondialdehyde (MDA) contents, decreased glutathione (GSH), increased Nuclear Factor Kappa B (NF-κB) and pro-inflammatory cytokines Tumor Necrosis Factor Alpha (TNF-α) and interleukin-1beta (IL-1β) levels in testis homogenate. In contrast, zeolite significantly amended these deleterious effects, and the potential mechanism involved the downregulation of Nuclear Factor Kappa B (NF-κB), Tumor Necrosis Factor Alpha (TNF-α) and interleukin-1beta (IL-1β), restoring glutathione (GSH) and reducing malondialdehyde (MDA). Also, zeolite was associated with an increased rate of pregnancy. Our data suggested that oxidative stress and inflammation are responsible for Cd-induced testicular injury and co-administration of zeolite exerts a protective effect via NF-κB /TNF-α/IL-1β pathway.

Keywords: cadmium chloride, zeolite, testicular injury

RESUMO

O cádmio (Cd) é um poluente ambiental que afeta vários tecidos e órgãos, inclusive o testículo. Muitos estudos demonstraram que a toxicidade do Cd causa comprometimento testicular por meio de estresse oxidativo e ação inflamatória. Portanto, este estudo teve como objetivo demonstrar a toxicidade testicular do Cd e a ação protetora da zeólita contra os efeitos deletérios do cádmio. Ratos machos adultos receberam Cd em uma dose de 30mg/kg/dia por 28 dias consecutivos com ou sem zeólita, que foi administrada em uma dose de 100mg/kg/dia por 28 dias. Foram avaliados o peso dos testículos, os espermatozoides (contagem, motilidade e anormalidades), a testosterona sérica e o hormônio luteinizante (LH), as enzimas testiculares fosfatase ácida (ACP) e fosfatase alcalina (ALP), as citocinas inflamatórias fator de necrose tumoral alfa (TNF-α), interleucina-1beta (IL-1β) e fator nuclear Kappa B (NF-κB) e estresse oxidativo. Aqui, descobrimos que o cádmio causou alterações nas características dos

*Corresponding author: rabdelgaber.c@ksu.edu.sa
Submitted: September 14, 2023. Accepted: January 10, 2024.

espermatozoides, distúrbios nos hormônios sexuais, declínio nas enzimas testiculares, conteúdo elevado de malondialdeído (MDA), diminuição da glutationa (GSH), aumento do fator nuclear Kappa B (NF-κB) e níveis de citocinas pró-inflamatórias do fator de necrose tumoral alfa (TNF-α) e interleucina-1beta (IL-1β) no homogenato do testículo. Em contraste, a zeólita alterou significativamente esses efeitos deletérios, e o mecanismo em potencial envolveu a regulação negativa do Fator Nuclear Kappa B (NF-κB), do Fator de Necrose Tumoral Alfa (TNF-α) e da interleucina-1beta (IL-1β), restaurando a glutationa (GSH) e reduzindo o malondialdeído (MDA). Além disso, a zeólita foi associada a uma maior taxa de gravidez. Nossos dados sugerem que o estresse oxidativo e a inflamação são responsáveis pela lesão testicular induzida por Cd e que a administração conjunta de zeólita exerce um efeito protetor por meio da via NF-κB /TNF-α/IL-1β.

Palavras-chave: cloreto de cádmio, zeólita, lesão testicular

INTRODUCTION

According to the available surveys, male infertility accounts for 30% of infertility cases, and its prevalence in the general population is between 9 and 15%. Male infertility is emerging as a critical cause of infertility worldwide (Caroppo and Colpi 2023). Environmental contaminants can affect male reproductive organs of humans, leading to poor sperm quality and function, and this is attributed to their harmful effects on spermatogenesis, steroidogenesis, Sertoli cells, blood–testis barrier, and epididymis in addition to germ cell apoptosis and decreased Leydig cell viability (Selvaraju *et al*., 2021). One main factor that evokes male infertility is the reactive oxygen species, which stimulate oxidative damage of sperm lipid membranes, DNA injury by gene mutation and direct DNA destruction, mitochondrial dysfunction, and apoptotic cell death (Ritchie and Ko, 2021). The other important factor is inflammation, which is among the more common causes of male infertility (Dutta *et al*., 2021), since pro-inflammatory cytokines like TNF-α and IL-1β in the male urogenital tract cause cytokine-mediated infertility. Furthermore, inflammation has been linked to increased levels of reactive oxygen species and oxidative stress, both of which are impacting male fertility (Sarkar *et al*., 2011).

Cadmium ($CdCl_2$) is the most widespread global environmental pollutant, which exerts various toxic effects on many tissues and organs of humans and animals (Ali *et al*., 2023). Exposure to cadmium occurs from both agricultural and industrial sources. Cadmium toxicity may occur by ingesting contaminated food and water, inhalation, and cigarette smoking (Genchi *et al*., 2020). Cadmium ($CdCl_2$) is a source of human toxicity due to its ability to accumulate in organs, especially testis (Li *et al*., 2016; Chen *et al*., 2022a). Also, the testis has extreme sensitivity to Cd toxicity, which impairs sperm quality and can reduce male fertility (Adamkovicova *et al*., 2016; Bhardwaj *et al*., 2021). This is attributed to its oxidative stress, inflammatory, and apoptotic effect (Fouad *et al*., 2009). So, using anti-oxidative and anti-inflammatory agents effectively protects against cadmium intoxication (Bashir *et al*., 2019). In addition, there is a strong link between testicular damage by cadmium and increased expression of malondialdehyde (MDA), a marker of oxidative stress and depletion of glutathione (GSH) and up-regulated levels of the pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) (Li *et al*., 2016).

Zeolites are natural or synthetic crystalline alumino-silicates with ion-exchanging properties used as farm animals feed additives for many years (Taş *et al*., 2007). In addition, zeolites are used in many fields, such as construction industries, aquaculture industries, agriculture, and space research (Simona and Camelia, 2019). Both natural and synthetic zeolites have been used in animal nutrition mainly to enhance performance and are effective in preventing ammonia and heavy metal toxicities (Papaioannou *et al*., 2005). Because it is an excellent detoxifying, antioxidant, and anti-inflammatory agent, it is employed in various industrial applications ranging from environmental cleanup to *in-vivo* oral usage in people as dietary supplements or medical devices (Mastinu *et al*., 2019).

Clinoptilolite, a natural zeolite crystal, has an antioxidant effect by reducing oxidant activity

and supporting antioxidant response (Saribeyoglu *et al*., 2011; Hcini *et al*., 2018). As well as micronized zeolites have antioxidant activities and may be useful as a modulator of oxidative stress in smokers (Atitlan-Gil *et al*., 2017).

Finally, our study aimed to evaluate the ameliorative effect of zeolite against cadmium chloride induced testicular toxicity.

MATERIALS AND METHODS

In this study, we used adult male Sprague-Dawley rats weighing 200±20g (4 months' age). They were obtained from the breeding colony and then maintained at the animal house of the Egyptian Drug Authority (EDA, Giza, Egypt), Formerly NODCAR. Animals received a standard diet, and water was allowed ad libitum during the study. An adaptation period of 2 weeks was given to animals in the animal house before the beginning of the experiment. All animals were kept at 21–24˚C and 40–60% relative humidity with a 12-h light–dark cycle.

Cadmium chloride was obtained from Sigma-Aldrich (St. Louis, MO), dissolved in distilled water, and given daily with a dose (30mg/kg/day Orally) according to a previous study by Wakeel *et al*. (2020) and pilot study for 28 days. Micronized zeolite was obtained from (Gongyi-Xiangrui Eco Material Co. Ltd, Gongyi, China) and was dissolved in 1% tween^{80} and given daily for 28 days with a dose (100mg/kg/day) following previous research (Saribeyoglu *et al*., 2011). All other chemicals were commercially available and of the highest purity and analytical grade.

Sixty rats were randomly allocated into five groups (n=12/group).

Group I: Animals served as normal control and received 1 ml of distilled water orally for 28 days.

Group II: Animals served as vehicle control and were treated with 1 ml of 1% tween^{80} per oz for 28 days.

Group III: Animals served as cadmium-treated group and were treated with $CdCl_2$ dissolved in distilled water (30 mg/kg/day; orally) for 28 days.

Group IV: Animals received zeolite dissolved in 1% tween^{80} in a 100 mg/kg/day dose orally for 28 days.

Group V: Treated animals were given $CdCl_2$ dissolved in distilled water at 30mg/kg/day per oz with orally given zeolite dissolved in 1% tween^{80} in a 100 mg/kg/day dose for 28 days.

All rats were weighed before sampling, after which the animals were anesthetized, and blood samples were collected through retro-orbital puncture of the venous plexus to determine serum testosterone and luteinizing hormone (LH) concentrations. After that, animals were euthanized by decapitation, and testes and epididymis were immediately removed, washed with ice-cold saline, and cleaned from the adhering tissue. The left testis was fixed in 10% neutral buffered formalin for 24 h for histopathological examination. The right testes were cleaned, the capsule and debris were removed, divided into two parts, and stored at −80°C and specimens underwent washing and subsequent homogenization in phosphate buffered saline (PBS) (10%, w/v) (10mmol/L, pH 7.4) and homogenate was centrifuged at 10000 ×g for 10 min at 4°C to obtain supernatant. This homogenate facilitated the evaluation of oxidative and inflammatory parameters. The other 50% were mated with females to calculate the pregnancy rate. The females at the end of pregnancy (at the 20th) were sacrificed; all fetuses were weighed and then put in 10% formalin for the embryological parameter.

Seminal fluid collected quickly from epididymis after sacrificing each animal. Estimation of sperm motility and count performed in accordance with Bearden and Fuquay (1980). To assess sperm abnormalities for each rat, a droplet from seminal fluid was mixed with one drop of Eosin-Nigrosin stain to detect malformed sperms. Then, films were examined randomly per slide under x 40, and the percentage of sperm abnormalities was recorded.

Serum levels of testosterone and luteinizing hormone (LH) were estimated using a Rat Testosterone ELISA kit (My Bio-Source®,

USA) and Rat Luteinizing Hormone kit (My Bio-Source®, USA) according to the manufacturer's procedure.

For the determination of MDA, we use the Life Span MDA reagent Kit and Blue Gene GSH Elisa kit for the estimation of reduced glutathione GSH in testicular homogenate according to manufacturer instructions.

The Bio-Vision acid phosphatase activity colorimetric kit was used to measure ACP activity, and the Abnova alkaline phosphatase kit was used to assess ALP in testicular homogenate per the manufacturer instructions.

Bio-Source® rat TNFα Elisa Kit, Bio-Source®rat IL-1β Elisa Kit, and CUSABIO®, China Elisa Kit were used for quantitative detection of TNF-α, IL-1β, and NF-κB content, respectively, in testicular supernatant.

Samples were taken from the testicular tissue of rats in different groups and fixed in 10% neutral buffered formalin (dissolved in phosphate buffer saline) for 24h. Histopathological samples were prepared using the method described by Bancroft and Gamble (2008) and then examined by the light microscope.

Weighed fetuses were taken from formalin to measure their body and tail lengths, and after that, the pregnancy rate was calculated.

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

RESULTS

Zeolite ameliorated $CdCl_2$-Altered relative weight of testes, sperm count, motility, and abnormalities. The testicular dysfunction in rats was investigated by detecting the relative weight of testes and sperm parameters. The $CdCl_2$ group showed a significant decrease relative to the weight of testes, sperm count, and motility by 18.5, 58.44, and 63.2%, respectively, and an increase in the incidence of sperm abnormalities by 164.33% when compared to the normal control group ($P<0.05$). On the other hand, zeolite administration significantly increased sperm count and motility by 98.9 and 87.1%, respectively; the relative weight of testes reached near the average values and decreased sperm abnormalities by 40.5% versus the cadmium group ($P<0.05$). At the same time, zeolite alone did not cause any change in the relative weight of testes, sperm count, and abnormalities. Still, it caused a significant decrease in sperm motility compared to normal control. The above-mentioned data explain zeolite's efficacy in mitigating $CdCl_2$-induced testicular injury in rats (Fig. 1,2).

Parallel to the observed attenuation in testicular function, administration of cadmium chloride exhibited a significant decrease in serum testosterone and LH concentrations by 69.5 and 72.5%, respectively, compared with the normal control group ($P<0.05$). Also, zeolite alone caused a significant reduction in serum testosterone and LH compared to the normal control group ($P<0.05$). However, zeolite co-treatment enhanced serum testosterone and LH production compared to $CdCl_2$-treated rats ($P<0.05$) (Fig. 3).

Next, we assessed testicular function markers as a second testicular injury measure. Rats administered with $CdCl_2$ displayed a significant decrease in ACP and ALP testicular activities by 85.9 and 77.7%, respectively, and zeolite alone also caused a significant decrease in ACP compared to the normal control group ($P<0.05$). Rats cotreated with zeolite significantly ameliorated ACP and ALP levels compared to the group that received $CdCl_2$ treatment only ($P<0.05$) (Fig. 3).

Oxidative stress plays a critical role in testicular dysfunction. To explore the oxidative stress status, we measured the testicular contents of MDA and glutathione (GSH). As expected, cadmium chloride caused a considerable increase in MDA levels by nearly 4-fold compared to the normal control ($P<0.05$). It induced a significant depletion in GSH, reaching 77.07% of the normal control level (Fig. 4). Treatment of animals with zeolite restored the normal levels of MDA by 44.6% and enhanced the GSH level by 110% when compared to the cadmium chloride group ($P<0.05$). Zeolite alone did not affect

oxidative stress biomarker levels compared to the normal control.

NF-κB, TNF-α, and IL-1β are critical mediators in the inflammatory response and are thought to enhance many pathophysiological alterations associated with inflammation. In the present study, the levels of TNF-α, IL-1β, and NF-κβ were detected in the testis. Exposure to $CdCl_2$ caused significant increases in the levels of NF-κβ, TNF-α, and IL-1β compared with those observed in the normal control ($P<0.05$). Meanwhile, Zeolite co-administration significantly decreased the levels of NFκβ, TNF-α, and IL-1β compared with the group that received $CdCl_2$ treatment only ($P<0.05$) (Fig. 5).

The histological assessment of the testicular specimen of control rats revealed seminiferous tubules and interstitial tissue; each tubule was lined with stratified epithelium (spermatogenic cells). Healthy spermatogenic cells, including spermatogonia, spermatocytes, spermatids, and spermatozoa, were present. Also, we noticed the presence of interstitial Leyding cells. Nevertheless, for rats that received $CdCl_2$, testes tissue had disorganized seminiferous tubules with a significant loss in the spermatogenic cell lineage, considerable damage to their architecture, disruption of the basement membrane, and there were no spermatozoa in its lumen but contained injured spermatogenic cells (Sloughed germ cells). In the inter-tubular connective tissue, there was a decrease in interstitial cells, an increase in interstitial width, and a congested interstitial blood vessel. These histopathological changes in the testicular tissue were significantly attenuated by zeolite as evidenced by the usual appearance of testicular tissue, seminiferous tubules with complete spermatogenesis, and entire germinal epithelial series (spermatogonia, spermatocytes, spermatid, and spermatozoa) (Fig. 6).

Six control males were mating with 12 females (after 28 days of their water receiving) to calculate their pregnancy rate for two weeks. Only four males were mated and caused pregnancy, with a percentage of 66.6%. For the tween 80 group, only three males from 6 males cause pregnancy (50%). Also, the zeolite pregnancy rate is 50% (3 males cause pregnancy). In the cadmium group, only about 33.33% is their pregnancy rate (2 males cause pregnancies). On the other hand, zeolite administration with cadmium increases the rate to 66.66% (4 male cause pregnancies) and increases the fertility of males.

Cadmium administration did not significantly change fetal body weight, body length, and tail length. Still, it showed a slight decrease in weights by 1.7%, body length by 1.4%, and tail length by 2.2% compared to the control group ($P<0.05$). The treated group with ($CdCl_2$+ Zeolite) also did not produce any significance but showed slight increases in the total fetal body weight by 0.5%, body length by 1.4%, and tail length by 1.4% compared to the cadmium group ($P<0.05$). Compared with the control group, treatment with Zeolite alone did not significantly alter fetal body weight, body length, and tail length (Table 1).

Table 1. Fetal body weight, body length, and tail length

Groups	Fetal body weight (gm)	Fetal body length (cm)	Fetal Tail length (cm)
Normal control	3.93± 0.04	5.63±0.09	1.41±0.03
$Tween^{80}$	3.92 ±0.05	5.61±0.08	1.41±0.03
Zeolite	3.91±0.04	5.61±0.07	1.40±0.05
$CdCl_2$	3.86±0.04	5.55±0.07	1.38±0.04
$CdCl_2$+ Zeolite	3.88±0.03	5.58±0.06	1.40±0.03

No significance from Normal control or $CdCl_2$ groups, respectively

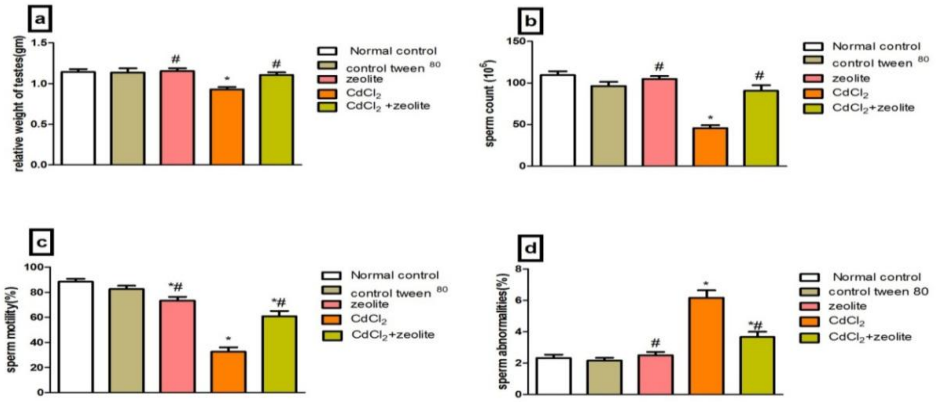

Figure 1. Effect of zeolite administration on **(a)** relative weight of testes **(b)** sperm count, **(c)** sperm motility, and **(d)** sperm abnormalities in rats challenged with cadmium. Values are presented as mean ± SE. * or # statistically significant from the control or cadmium group, respectively

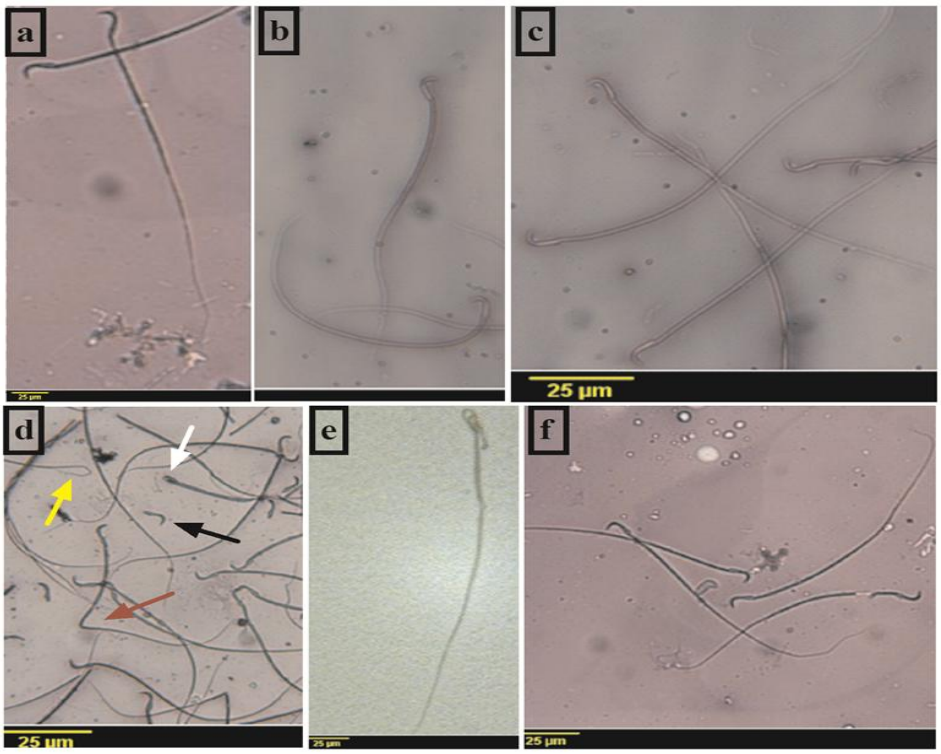

Figure 2. Photomicrographs of smear from epididymal fluid stained by eosin & nigrosin stain (×40) from the rat in **(a)** normal control group, **(b)** tween^{80} group and **(c)** zeolite group showing normal sperm formation with head, neck and tail. Cadmium chloride group showing **(d)** Bent mid piece (red arrow), looped tail (yellow arrow), amorphous head (white arrow), detached tail (black arrow) and **(e)** abnormal head **(f)** Cadmium and zeolite co-treated group showing normal sperm formation with head, neck and tail.

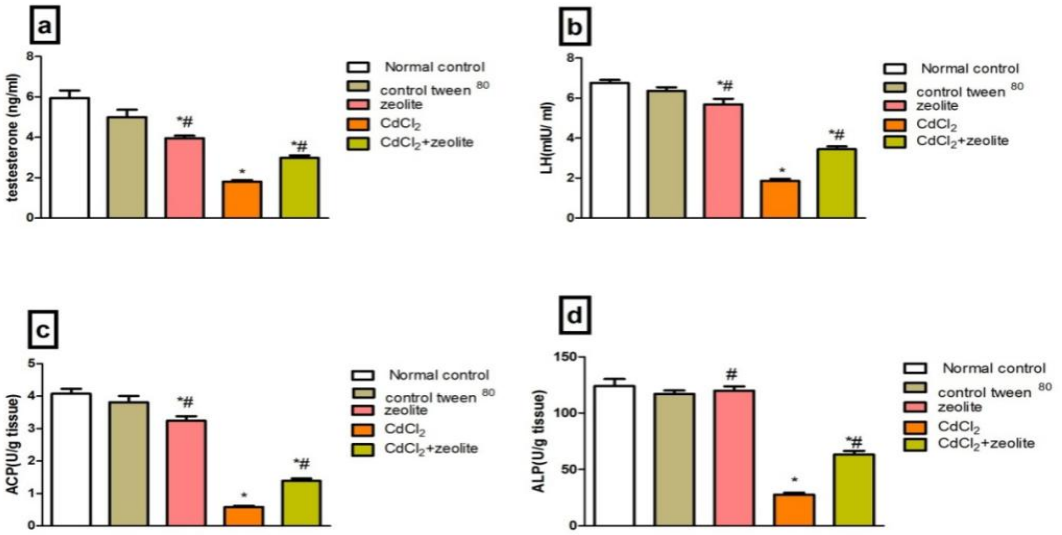

Figure 3. Effect of zeolite administration on **(a)** testosterone, **(b)** LH, **(c)** ACP, and **(d)** ALP on rats challenged with cadmium. Values are presented as mean ± SE. * or # statistically significant from the control or cadmium group, respectively

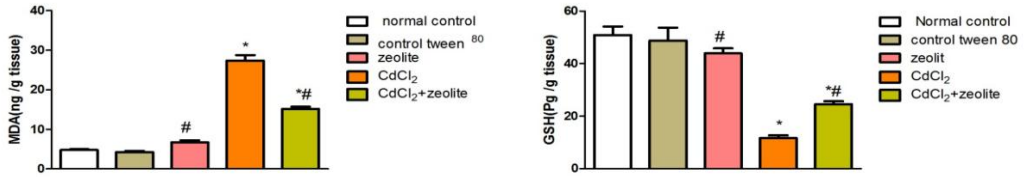

Figure 4. Effect of zeolite administration on **(a)** MDA and **(b)** GSH on rats challenged with cadmium. Values are presented as mean ± SE. * or # statistically significant from the control or cadmium group, respectively.

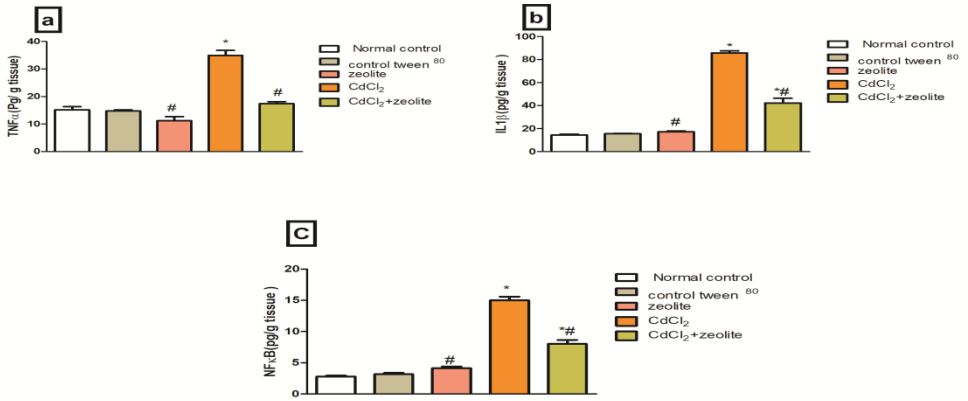

Figure 5. Effect of zeolite administration on **(a)** TNF-α, **(b)** IL-1β, and **(c)** NF-κB on rats challenged with cadmium. Values are presented as mean ± SE. * or # statistically significant from the control or cadmium group, respectively

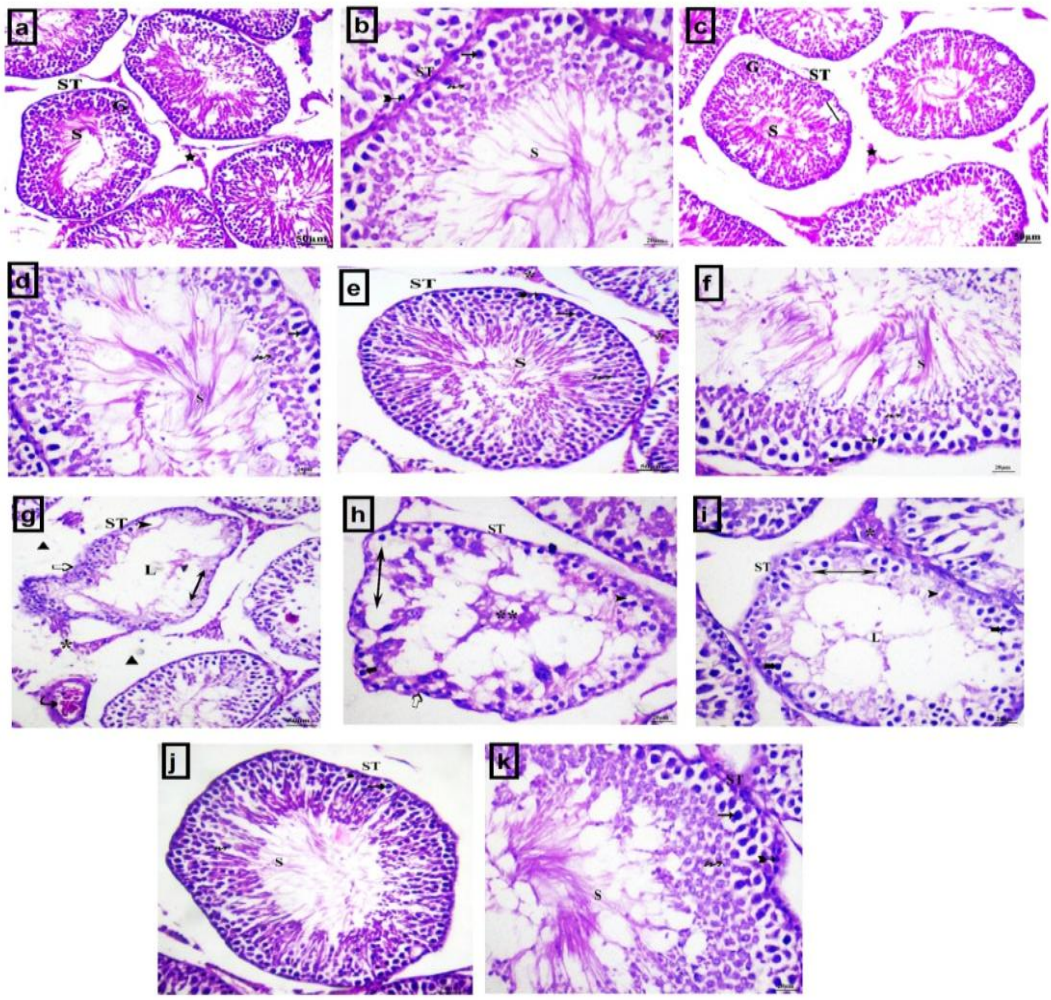

Figure 6. Photomicrographs of testicular tissue (H&E) of **(a)**, **(b)** control rat, **(c)**, **(d)** tween^{80} treated rat and **(e)**, **(f)** zeolite treated rat showing normal histological structure of the mature active seminiferous tubules(ST) with sperm (S) in lumen, germinal cells (G) spermatocyte (Thin arrow), spermatid (Zigzag arrow) and spermatogonia (Bifid arrow), **(g),(h)**, **(i)** cadmium treated rat showing irregular and undulating membrane (hollow arrow), absence of spermatozoa in lumen (L), loss of germinal epithelium series (Double head arrow), congested blood vessel (Curved arrow), vacuolation (Head arrow) and lumen with damaged cells (Double asterisk). And **(j)**, **(k)** cadmium+ zeolite treated rat showing the normal histological structure of the mature active seminiferous tubules (ST) with sperm (S) in the lumen, spermatocyte (Thin arrow), spermatid (Zigzag arrow) and spermatogonia (Bifid arrow).

DISCUSSION

Reactive oxygen species in limited amounts are necessary for cell function but excessive reactive oxygen species production is known as oxidative stress which is responsible for the pathogenesis of several diseases (Pisoschi *et al*., 2021). In addition, Oxidative stress is harmful to male fertility as it causes oxidative damage to reproductive cells (Dutta *et al*., 2021), negatively affects spermatozoa quality and their fertilizing capability (Tvrda *et al*., 2011).

Deleterious effects of cadmium are related to the production of reactive oxygen species (ROS) as ROS production, glutathione depletion, and lipid peroxidation are mechanisms by which cadmium exerts its actions (Branca *et al*., 2020). Cd also provokes oxidative stress, and spermatogenic cell apoptosis, and reduces androgen production and sperm functions (Bhardwaj *et al*., 2021). In our

investigation, Cadmium enhanced MDA level and caused the depletion of glutathione (GSH) as mentioned by Li *et al*. (2016). So cadmium has a direct oxidative stress effect (Zhu *et al*., 2020). On the other hand, this oxidative stress is mitigated by zeolite which reduces oxidant activity and supports antioxidant response via decreasing the level of MDA and increasing the level of glutathione (GSH) as described by Saribeyoglu *et al*. (2011) and Wu *et al*. (2013).

Excessive ROS production modulates proteins and genes, so it can lead to inflammatory diseases (Chatterjee, 2016), and activate NF-KB (Siomek, 2012), which regulates the initiation of inflammation (Sanz *et al*., 2010). Binding sites for the transcriptional regulatory factor nuclear factor kappa B (NF-κB) are present in the promoter regions of many of the proinflammatory cytokines, so increased activation of NF-κB leads to enhanced pro-inflammatory mediators' expression (Abraham, 2000).

Cadmium also exerts its toxic effect by evoking inflammation via activation of the NF-κB/ TNF-α pathway and via increasing the expression of IL-1β (Chen *et al*., 2022b). This is in line with our results as we found that cadmium caused a considerable increase in TNF-α, IL-1β, and NF-κB as NF-κB is a master regulator for inflammatory processes in response to both injury and infection (Napetschnig and Wu, 2013; Fouad *et al*., 2020). Zeolite amended this inflammation due to its anti-inflammatory effect (Petkov *et al*., 2021), through the decrease of NF-κB, TNF-α, and IL-1β levels as mentioned in the previous study (Yapislar *et al*., 2016), as down-regulation of NF-κB contributes to the pathogenic processes of various inflammatory diseases.

The decline in serum testosterone and LH is a prominent sign of cadmium toxicity. In our investigation, cadmium evoked a valuable decrease in testosterone levels. Likewise, earlier research indicated that cadmium caused a decline in testosterone levels (Almeer *et al*., 2018; Fouad *et al*., 2020). This may be attributed to its cytotoxic effect on Leydig cells, the primary source of testosterone, leading to functional disturbance of Leydig cells (Pavlova and Atanassova, 2018).

Also, cadmium caused a significant drop in LH levels, and this finding is in line with Lafuente *et al*. (1999); this deteriorative effect was initiated by a disruption of the regulatory mechanisms of the hypothalamic–pituitary–testicular axis by cadmium (Lafuente *et al*., 2000). Cadmium affects the hypothalamic-pituitary-testicular axis by two different pathways; the first one by its effect on Leydig cells and the other one by affecting the release of noradrenalin, a regulator of hypothalamus hormone secretion, which resulted in changes in plasma testosterone and LH levels (Lafuente *et al*., 2003). On the other hand, we noticed that zeolite enhanced testosterone and LH levels and overcome endocrine toxicity caused by cadmium.

An important marker for testicular dysfunction is the impairment of sperm characteristics as we found that cadmium intoxication caused a decrease in sperm count, and motility as well as increased sperm abnormalities, and this was confirmed in previous studies (Arab *et al*., 2022), depending on the fact that testosterone is necessary for spermatogenesis (Griswold, 1998). This decline in sperm count may be due to the decline in testosterone level by cadmium as there is a strong relation between testosterone level and sperm count (Salman *et al*., 2013). Sperm is highly susceptible to oxidative stress created by cadmium causing axonemal damage and increased mid-piece morphological defects which reduce sperm motility (Kurkowska *et al*., 2020). Finally, the increase of TNF-α and IL-1β by cadmium may be a reason for disturbance in sperm characters as many pro-inflammatory cytokines such as TNF-α and IL-1β increase the level of lipid peroxidation of sperm membranes to levels that can affect the sperm fertility capacity (Martinez *et al*., 2007; Paira *et al*., 2022). As endogenous inflammation increases ROS levels, which might induce sperm oxidative damage and sperm motility, one of the most sensitive indicators of this damage (Shi *et al*., 2012).

Zeolite also abolished these deleterious effects and enhanced sperm quality and characteristics, and this result was consistent with Mohammed *et al*. (2021) and correlated to its ability to reduce lipid peroxidation by scavenging free radicals (Atitlan-Gil *et al*., 2017), and to oppose inflammation (Qi *et al*., 2021).

One of the signs of cadmium toxicity is the decline in testicular markers (ACP and ALP) as these testicular enzymes decreased in Cd-treated rats as mentioned in earlier research (Ola-Mudathir *et al*., 2008; El-Maraghy and Nassar 2011). This decrease in the ACP is thought to be due to the reduction of testicular steroidogenesis, and the decline in ALP may be due to a decline in the testicular Zn^{2+} level, which is an essential part of the enzyme activity (El-Maraghy and Nassar 2011), as Cd can displace zinc (Ebrahimi, 2006). Finally, zeolite abrogated Cd-induced decrease in ACP and ALP by competing for its toxicity as zeolite can prevent heavy metal toxicities similar to previous publications (Papaioannou *et al*., 2005).

Our histopathological findings revealed that $CdCl_2$ intoxication leads to significant structural alterations in the testes compared to controls as disorganization of seminiferous tubules, a significant loss in the spermatogenic cell, and decreased spermatozoa production, a decrease in interstitial cells and congested interstitial blood vessel as mentioned in previous publications (Kara *et al*., 2007; Wang *et al*., 2020). This histopathological alteration may be attributed to oxidative stress produced by $CdCl_2$ that caused depletion of antioxidant defenses and increased lipid peroxidation in testicular tissue (Amara *et al*., 2008; Momeni and Eskandarir 2020; Han *et al*., 2020).

We found that zeolite can repair these injuries in testicular tissue, and this is related to its antioxidant effect as zeolite can protect the cells from ROS-induced cell death and reduce mitochondrial ROS production (Montinaro *et al*., 2013).

It is important to address the potential limitations of our study. We did not measure 3β-hydroxysteroid-dehydrogenase and 17β-hydroxysteroid-dehydrogenase as playing vital role in testosterone biosynthesis. Another main limitation that serum level of follicle-stimulating hormone (FSH) was not measured as FSH regulates spermatogenesis. Finally, some transcriptional factors as Nrf2 and its downstream were not evaluated and it plays important role in synthesis of antioxidants.

CONCLUSIONS

Cd can induce oxidative stress and inflammation in rat testes. Zeolite antagonizes Cd toxicity in rat testes by reducing oxidative stress and inhibiting NF-κB /TNF-α/IL-1β pathway. This study revealed the specific mechanism of Zeolite against Cd and provided a new guide to reduce Cd toxicity.

ACKNOWLEDGMENT

The authors thank Prof. K.A. Ahmed (Faculty of Veterinary Medicine, Cairo University) for her careful assistance in histopathological examination.

FUNDING

This study was supported by the Researchers Supporting Project (RSP2024R25), King Saud University, Riyadh, Saudi Arabia.

INSTITUTIONAL REVIEW BOARD STATEMENT

Experimental procedures were applied following the ethical guidelines for investigations in laboratory animals and were approved by the standard guidelines of EDA (Approval number: NODCAR/1/17/2021) in handling the experimental animals and conforms to the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

REFERENCES

ABRAHAM, E. NF-κB activation. *Crit. Care. Med.,* v.28, p.100-104, 2000.

ADAMKOVICOVA, M.; TOMAN, R.; MARTINIAKOVA, M. *et al.* Sperm motility and morphology changes in rats exposed to cadmium and diazinon. *Reprod. Biol. Endocrinol.,* v.14, p.42, 2016.

ALI, W.; BIAN, Y.; ALI, H. *et al*. Cadmium-induced impairment of spermatozoa development by reducing exosomal-MVBs secretion: a novel pathway. *Aging*, v.15, p.4096-4107, 2013.

ALMEER, R.S.; SOLIMAN, D.; KASSAB, R.B. *et al*. Royal jelly abrogates cadmium-induced oxidative challenge in mouse testes: involvementof the Nrf2 pathway. *Int. J. Mol. Sci.*, v.19, p.3979-3995, 2018.

AMARA, S.; ABDELMELEK, H.; GARREL, C. *et al.* Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. *J. Reprod. Dev.*, v.54, p.129-134, 2008.

ARAB, H. H.; ELHEMIELY, A.A.; EL-SHEIKH, A.A.K. *et al.* Repositioning linagliptin for the mitigation of cadmium-induced testicular dysfunction in rats: targeting HMGB1/TLR4/NLRP3 axis and autophagy. *Pharmaceuticals*, v.15, p.852, 2022.

ARMITAGE, P.; BERRY, G. *Comparison of several groups*. Oxford: Blackwell Scientific Publications, 1987. p.186-213.

ATITLAN-GIL, A.; LOZA, M.M.B.; JIMÉNEZ-ORTEGA, J.C. *et al.* Activated and micronized zeolite in the modulation of cellular oxidative stress in Mexican smokers: a randomized clinical trial. *Rev. Inves. Clin*., v.69, p.146-151, 2017.

BANCROFT, J.D.; GAMBLE, M. *Theory and practice of histological techniques*. 6.ed. Londres: Elsevier Health Sciences, 2008.

BASHIR, N.; SHAGIRTHA, K.; MANOHARAN, V.; MILTONPRABU, S. The molecular and biochemical insight view of grape seed proanthocyanidins in ameliorating cadmium-induced testes-toxicity in rat model: implication of PI3K/Akt/Nrf-2 signaling. *Biosci. Rep,*. v.39, n.1, 2019.

BEARDEN, H.J.; FUQUAY, J.W. *Applied animal reproduction*. Reston, Virginia: Reston Publishing Company, Inc. 1980. 337p.

BHARDWAJ, J.K.; PANCHAL, H.; SARAF, P. Cadmium as a testicular toxicant: a review. *J. Appl. Toxicol.*, v.41, p.105-117, 2021.

BRANCA, J.J.V.; FIORILLO, C.; CARRINO, D. *et al*. Cadmium-induced oxidative stress: focus on the central nervous system. *Antioxidants*, v.9, p.492-513, 2020.

CAROPPO, E.; COLPI, G.M. Male Infertility: a review of key papers appearing in the reproductive medicine and andrology section of the journal of clinical medicine. *J. Clin. Med.*, v.12, p.2366, 2023.

CHATTERJEE, S. Oxidative Stress, Inflammation, and Disease. DZIUBLA, T.; BUTTERFIELD, D.A. *Oxidative stress and biomaterials.* Amsterdam: Elsevier, 2016. p.35-58.

CHEN X.; BI, M.; YANG J.; CAI, J. *et al*. Cadmium exposure triggers oxidative stress, necroptosis, Th1/Th2 imbalance and promotes inflammation through the TNF-α/NF-κB pathway in swine small intestine. *J. Hazard. Mater.*, v.421, p.126704, 2022a.

CHEN, Z.; ZUO, Z.; CHEN, K. *et al*. Activated Nrf2 pathway by vitamin E to attenuate testicular injuries of rats with sub-chronic cadmium exposure . *Biol. Trace. Elem. Res.*, v.200, p.1722-1735, 2022b.

DUTTA, S.; SENGUPTA, P.; SLAMA, P.; ROYCHOUDHURY, S. Oxidative stress, testicular inflammatory pathways and male reproduction. *Int. J. Mol. Sci.*, v.22, p.10043, 2021.

EBRAHIMI, M. Competition of zinc, cadmium and calcium for binding sites in sperm of trout and carp. *Iran. J. Vet. Res*., v.7, p.70-76, 2006.

EL-MARAGHY, S.A.; NASSAR, N.N. Modulatory effects of lipoic acid and selenium against cadmium-induced biochemical alterations in testicular steroid genesis. *J. Biochem. Mol. Toxicol*., v.25, p.15-25, 2011.

FOUAD, A.A.; ABDEL-AZIZ, A.M.; HAMOUDA, A.A.H. Diacerein downregulates NLRP3/Caspase-1/IL-1β and IL-6/STAT3Pathways of inflammation and apoptosis in a rat model of cadmium testicular toxicity. *Biol. Trace Elem. Res.*, v.195, p.499-505, 2020.

FOUAD, A.A.; QURESHI, H.A.; AL-SULTAN, A.I.; YACOUBI, M.T.; ALI, A.A. Protective effect of hemin against cad-mium-induced testicular damage in rats. *Toxicology*, v.153, p.153-160, 2009.

GENCHI, G.; SINICROPI, M.S.; LAURIA, G.; CAROCCI, A.; CATALANO, A. The Effects of Cadmium Toxicity. *Int. J. Environ. Res. Public Health*, v.17, p.3782-3806, 2020.

Griswold, M.D. The central role of sertoli cells in spermatogenesis. *Semin. Cell Dev. Biol*., v.9, p.411-416, 1998.

HAN, C.; ZHU, Y.; YANG, Z. *et al*. Protective effect of Polygonatumsibiricum against cadmium-induced testicular injury in mice through inhibiting oxidative stress and mitochondria-mediated apoptosis. *J .Ethnopharmacol*., v.261, p.113060, 2020.

HCINI, E.; BEN SLIMA, A.; KALLEL, I. *et al*. Does supplemental zeolite (clinoptilolite) affect growth performance, meattexture, oxidative stress and production of polyunsaturated fatty acid of Turkey poults?. *Lipids Health Dis.*, v.17, p.177-185, 2018.

KARA, H.; CEVIK, A.; KONAR, V.; DAYANGAC, A.; YILMAZ, M. Protective effects of antioxidants against cad-mium-induced oxidative damage in rat testes. *Biol. Trace Elem. Res.*, v.120, p.205-211, 2007.

KURKOWSKA, W.; BOGACZ, A.; JANISZEWSKA, M. *et al*. Oxidative stress is associated with reduced sperm motility in normal semen. *Am. J. Mens. Health*, v.14, n.5, 2020.

LAFUENTE, A.; CANO, P.; ESQUIFINO, A. Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? *Biometals*, v.16, p.243-250, 2003.

LAFUENTE, A.; MÁRQUEZ, N.; PÉREZ-LORENZO, M.; PAZO, D.; ESQUIFINO, A.I. Pubertal and postpubertal cadmium exposure differentially affects the hypothalamic–pituitary–testicular axis function in the rat. *Food Chem. Toxicol.*, v.38, p.913-923, 2000.

LAFUENTE, A.; MÁRQUEZ, N.; PIQUERO, S.; ESQUIFINO, A. I. Cadmium affects the episodic luteinizing hormone secretion in male rats: possible age-dependent effects. *Toxicol. Lett.*, v.104, p.27-33, 1999.

LI, R.; LUO. X.; LI, L. *et al*. The protective effects of melatonin against oxidative stress and inflammation induced by acute cadmium exposurenin mice testis. *Biol. Trace Elem. Res.*, v.170, p.152-164, 2016.

MARTINEZ, P.; PROVERBIO, F.; CAMEJO, M.I. Sperm lipid peroxidation and pro-inflammatory cytokines. *Asian J. Androl.*, v.9, p.102-107, 2007.

MASTINU, A.; KUMAR, A.; MACCARINELLI, G. *et al*. Zeolite clinoptilolite: therapeutic virtues of an ancient mineral. *Molecules*, v.24, p.1517-1532, 2019.

MOHAMMED, A.K.; KHALIL, W.A.; YOUSSEF, H.F. *et al*. Influence of adding zeolite loaded with different charges to semen extender on sperm quality in rabbits after cryopreservation. *Cryobiology*, v.103, p.107-115, 2021.

MOMENI, H.R.; ESKANDARI, N. Curcumin protects the testis against cadmium-induced histopathological damages and oxidative stress in mice. *Hum. Exp. Toxicol*., v.39, p.653-661, 2020.

MONTINARO, M.; UBERTI, D.; MACCARINELLI, G. *et al*. Dietary zeolite supplementation reduces oxidative damage and plaque generation in the brain of an Alzheimer's disease mouse model. *Life Sci*., v.92, p.903-910, 2013.

NAPETSCHNIG, J.; WU, H. Molecular basis of NF-κB signaling. *Annu. Rev. Biophys.*, v.42, p.443-468, 2013.

OLA-MUDATHIR , K.F.; SURU, S.M.; FAFUNSO, M.A.; OBIOHA, U.E.; FAREMI, T.Y. Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats . *Food Chem. Toxicol.*, v.46, p.3604-3611, 2008.

PAIRA, D.A.; SILVERA-RUIZ, S.; TISSERA, A. *et al*. Interferon γ, IL-17, and IL-1β impair sperm motility and viability and induce sperm apoptosis. *Cytokine*, v.152, p.155834, 2022.

PAPAIOANNOU, D.; KATSOULOS, P.D.; PANOUSIS, N.; KARATZIAS, H. The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: a review. *Microporous Mesoporous Mater.*, v.84, p.161-170, 2005.

PAVLOVA, E.; ATANASSOVA, N. Impact of cadmium on male fertility. *Acta Morphol. Anthropol.*, v.25, p.108-116, 2018.

PETKOV, V.; SCHÜTZ, B.; EISENWAGEN, S.; MUSS, C.; MOSGOELLER, W. PMA-zeolite can modulate inflammation associated markers in irritable bowel disease – an explorative randomized double blinded, controlled pilot trial. *Neuro Endocrinol. Lett.*, v.42, p.1-12, 2021.

PISOSCHI, A.M.; POP, A.; IORDACHE, F. *et al*. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *Eur. J. Med. Chem*., v.209, p.112891, 2021.

QI, Y.; QIAN, K.; CHEN, J. *et al*. thermoreversible antibacterial zeolite-based nanoparticles loaded hydrogel promotes diabetic wound healing via detrimental factor neutralization and ROS scavenging. *J. Nanobiotechnol.*, v.19, p.414, 2021.

RITCHIE, C.; KO, E.Y. Oxidative stress in the pathophysiology of male infertility. *Andrologia*, v.53, p.3581, 2021.

SALMAN, T.M.; ALAGBONSI, I.A.; OLAYAKI, L.A. *et al*. Honey increases sperm count in male albino rats by enhancing testosterone production. *Biokemistri*, v.25, p.39-44, 2013.

SANZ, A.B.; SANCHEZ-NIN, M.D.; RAMOS, A. *et al*. NF-kappaB in renal inflammation. *J. Am. Soc. Nephrol., 21*, v.21, p.1254-1262, 2010.

SARIBEYOGLU, K.; AYTAC, E.; PEKMEZCI, S. *et al*. Effects of clinoptilolite treatment on oxidative stress after partial hepatectomy in rats. *Asian J. Surg.*, v.34, p.153-157, 2011.

SARKAR, O.; BAHRAINWALA, J.; CHANDRASEKARAN, S. *et al*. Impact of inflammation on male fertility. *Front. Biosci.*, v.3, p.89-95, 2011. (Elite edition).

SELVARAJU V.; BASKARAN, S.; AGARWAL, A.; HENKEL, R. Environmental contaminants and male infertility: Effects and mechanisms. *Andrologia*, v.53, p.13646, 2021.

SHI, T.Y.; CHEN, G.; HUANG, X. *et al*. Effects of reactive oxygen species from activated leucocytes on human sperm motility, viability and morphology. *Andrologia*, v.44, p.696-703, 2012.

SIMONA, M.; CAMELIA, T. *Zeolites applications in veterinary medicine*. Zeolites - new challenges. [s.l.]: Intechopen, 2019. chap.7, p.123-129.

SIOMEK, A. NF-κB signaling pathway and free radical impact. *Acta. Biochim. Pol*., v.59, p.323-331, 2012.

TAŞ, M.; DEMIREL, R.; ŞENTÜRK, D. *et al*. Effects of dietary natural zeolite on the testicular Weight, body weight and spermatological characteristics in rats. *J. Fac. Vet. Med. Istanbul Univ.*, v.33, p.33-42, 2007.

TVRDA, E.; KNAZICKA, Z.; BÁRDOS, L.; MASSANYI, P.; LUKAC, N. Impact of oxidative stress on male fertility – a review. *Acta. Vet. Hung*., v.59, p.465-484, 2011.

WAKEEL, J.; EHSAN, N.; AKHTAR, R.W.; SHAH, S.A.H. Morphology, histopathology and hematology as biomarkers of cadmium toxicity in field rats. *Iran. J. Toxicol.*, v.14, p.33-42, 2020.

WANG, J.; ZHU, H.; WANG, K.; YANG, Z.; LIU, Z. Protective effect of quercetin on rat testes against cadmium toxicityby alleviating oxidative stress and autophagy. *Environ. Sci. Pollut. Res Int.*, v.27, p.25278-25286, 2020.

WU, Y.; WU, Q.; ZHOU, Y.; AHMAD, H.; WANG, T. Effects of clinoptilolite on growth performance and antioxidant status in broilers. *Biol. Trace Elem. Res.*, v.155, p.228-235, 2012.

YAPISLAR, H.; TASKIN, E.; OZDAS, S.; AKIN, D.; SONMEZ, E. Counteraction of apoptotic and inflammatory effects of adriamycin in the liver cell culture by clinopitolite. *Biol. Trace Elem. Res.*, v.170, p.373-381, 2016.

ZHU, Q.; LI, X.; GE, R. Toxicological effects of cadmium on mammalian testis. *Front. Genet.*, v.11, p.527, 2020.