ISSN: 2455-7080

Contents lists available at http://www.albertscience.com

ASIO Journal of Experimental Pharmacology & Clinical Research (ASIO-JEPCR)

Volume 6, Issue 1, 2020, 41-53

METAL INDUCED TESTICULAR TOXICITY

Amit Gupta*

Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Raebareli, U.P., India.

ARTICLE INFO

Article History
Received: 23rd July, 2020
Accepted:26th July, 2020

Corresponding Author:
***Amit Gupta**
Email: amitfeb081@gmail.com
Mobile no.: 8077881026

† Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Raebareli, U.P., India.

ABSTRACT

Metal exposure leads to severe damage in male reproductive organs affecting male fertility. Although many of the metals are essential for various biological processes but on the other hand, these essential metals become toxic with the increasing dose and exposure. The primary objective of this review article is to find out the various metals induced toxicity on the reproductive system of male, especially on testicular system. The main aim is to collect the all data in a cumulative way and to compile all of these and represent them in a tabulated form. The overall result of this review offer a confirmation that certain transition metals such as As, Cd, Cr, Hg, Mn, Mo, Pb, Ni, V and Zn may adversely affect male reproductive functions such as spermatogenesis, sperm quality, secretory functions of accessory glands, libido, fertility, serum testosterone level and antioxidant defense system. Most of the reports on the reproductive toxicity of transition metals are from experimental animal studies.

Keywords: Metal toxicity, reproductive system, testicular toxicity, spermatogenesis.

INTRODUCTION:

During the past 50 years, thousands of metals and chemicals have been released into the general environment [1]. It is often the case that the nonessential toxicant metals mimic essential metals and thereby gain access to and potentially disrupt key cellular functions. Due to the rapid industrialization and overgrowing urbanization, effects of metals on male reproductive system have become major health concern [2, 3]. This can also account for bioaccumulation of toxic metals [4, 5]. It is undeniable that good quality semen is essential for reproductive success. This quality appears to have been directly affected in recent years. Since 1990s, various authors have reaffirmed the possible significant drop in sperm quality and consequently an increase in male infertility rates [6, 7, 8].

Male infertility accounts to about 50% of infertility cases in 10-15% of couples [9]. One of the major factors associated with male infertility is the quantity and quality of sperm produced [10]. Failure of spermatogenesis is the result of several causes such as systemic diseases, endocrine disorders, malnutrition, genetic factors and environmental hazards [11]. Heavy metals may compromise male reproduction, as demonstrated by epidemiological and animal studies [12]. Lead poisoning causes inhibition of testicular functions along with those of the secondary sexual glands like the prostrate, epididymis and seminal vesicle, resulting in the alteration of their biochemical composition and affecting both steroidogenesis as well as gametogenesis [13]. Accumulation of lead in the testis is known to have anti-spermatogenic effect [14]. According to Anjum et al., the testis of lead treated rats revealed remarkable degeneration and atrophied seminiferous tubules with absence of regular differentiated stages of germ cells to mature spermatozoa [15].

Occupational exposure is associated with paint, plastic, glass, metal alloy production, and mining activities. Nonoccupational exposure can occur by the ingestion of food and water containing the metal and bioaccumulation in plants and aquatic organisms. Other forms of contamination are inhalation of cigarette smoke or air pollution caused by forest fires, mining areas, and metal refining industries. It is known that Cd exposure can affect organs, such as the liver, lung, and kidneys, and the testicles are particularly sensitive to toxicity mediated by this pollutant. Changes in testicular structure due to Cd intoxication include damage to germ and Sertoli cells, as well as degeneration and testicular necrosis, since this metal leads to the rupture of the blood-testis barrier. When ingested, Cd is absorbed in

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

the duodenum by the divalent metal receptor (DMT^{-1}), which absorbs microminerals. This competition may reduce the concentration of essential minerals, such as magnesium (Mg), iron (Fe), zinc (Zn), selenium (Se), and copper (Cu), which is important for the development and maintenance of spermatogenesis. The aforementioned minerals act as cofactors of antioxidant enzymes, such as superoxide dismutase, and a reduction of mineral levels increases reactive oxygen species (ROS) concentrations, which is also a mechanism of Cd toxicity [16-27].

Over the past few decades, male infertility has increased a phenomenon that is closely associated with environmental pollution. Particularly, cadmium is a highly toxic heavy metal that has been reported to induce male infertility. With the rise of the incidence rate of infertility, the adverse effects of environmental factors are becoming an increasing concern. Particularly, environmental pollution has posed a serious threat in recent years. Guangxi is an area with high cadmium, which has an adverse effect on the safety of life. Previous studies have demonstrated that the reproductive toxicity of cadmium is the highest, particularly in the male reproductive toxicity system. Human intake of cadmium primarily originates from the consumption of contaminated water, crops, cigarettes or other sources. Cadmium deposition in the kidney, bones, liver, lung and reproductive organs leads to severe organ impairment [28-39].

Transition metals are those elements whose atom has an incomplete d sub-shell, or which can give rise to cations with an incomplete d sub-shell. Because of this physical characteristic, transition metals have a wide variety of applications in the industrial world. They are the key to making different alloys, colored paints, photo reactive eye glasses, mercury thermometers and in medicines. Transition metals are also used as a catalyst in fertilizers and chemical industries. The characteristic property of transition metal is that they have many oxidation states, due to the relatively low reactivity of unpaired d electrons. The major role of transition metal ions is in oxidation-reduction reactions. In biological systems, transition metals are mostly conjugated or bound to proteins forming metalloproteins. Most of the metals in metalloproteins are part of enzymatic systems, have structural functions, or use the protein to be transported to their target site in the organism [40].

Transition metals include trace elements that are of significance for mammalian physiology like: (i) Cobalt (Co), a component of cobalamine, or vitamin B12, (ii) molybdenum (Mo), an electron transfer agent in enzymes such as xanthineoxidase and sulphite reductase, (iii) vanadium (V), which is biochemically related to glucose and lipid metabolism, (iv) copper (Cu), responsible for the production of a wide range of neurotransmitters, also required for the proper function of vitamin C and iron absorption and (v) zinc (Zn), necessary for a healthy immune system [41].

Certain transition metals like Zn, Cu, manganese (Mn), gold (Au) and nickel (Ni) play a significant role in male reproductive functioning and deficiency of these trace metals have negative impact on spermatogenesis and semen quality. Zinc is essential for the maintenance of germ cells, the progression of spermatogenesis, stabilization of the cell membrane and regulation of capacitation, acrosome reaction and sperm motility [42]. Its deficiency leads to gonadal dysfunction, decreases testicular weight, and causes shrinkage of seminiferous tubules [43]. Mn is a potent stimulator of sperm motility through the stimulation of adenylate cyclase activity [44]. It also stimulates luteinizing hormone (LH) secretion and spermatogenesis in pre-pubertal male rats and its deficiency can impair fertility and cause birth defects [45]. Gold also has been claimed to have a beneficial effect on testicular function and sperm. “Swarna bhasma” (ash of gold) has been used with good results by Ayurvedic practitioners in the treatment of infertility [46]. The role of Cu in male reproductive capacity appears to be largely unknown, but this metal appears to be involved in spermatozoa motility and it may also act at the pituitary receptors which control the release of LH [47]. Nickel is also an activator of some enzymes (dehydogenase and carboxylase). Nickel deficiency can have a negative impact on spermatogenesis and semen quality [48,49].

The incidence of chemically-induced infertility appears to be on the increase worldwide [50]. Therefore, the toxic effects of environmental toxins and drugs on the human reproductive system have become a major health concern [51,52]. Cadmium (Cd), a toxic heavy metal of both occupational and environmental concern, has found its relevance in several industrial processes such as in electroplating and manufacturing of paint pigments, batteries, plastic, and fertilizers. However, in regions of inadequate exposure control, it readily bioaccumulates in biological systems where it induces deleterious health effects [53]. When released into the environment following occupational activities, it is readily absorbed from the soil by the root of plants; making food consumption a major source of its exposure [54, 55]. It is readily absorbed by the body via oral route (by means of Cd-contaminated water) and inhalation (particular in cigarette smoke) [56]. Unlike most heavy metals, once absorbed by the body Cd exposure can induce deleterious effects at relatively lower doses [57-59]. Its toxic effects are expressed in the testes even before pathological changes occur in other organs [60], although its main repository organ is the kidney [61]. Cd is reputed to exert its toxic effects by inducing reactive oxygen species (ROS) generation through oxidative damage [62]. These ROS, mainly O_2+, H_2O_2 and OH+ [63], initiate reactions with cellular biomolecules, and consequently, results in lipid peroxidation, altered the antioxidant system, membrane protein damage, DNA damage, and apoptosis [64-65]. It was, therefore, hypothesized that a potent antioxidant could retard or inhibit the basic mechanism of Cd-induced deleterious alterations (generation of ROS) and possibly ameliorate its toxic effects in biological systems. This hypothesis was tested using dietary polyphenols which are relatively cheaper, readily available, and considered to have considerable lesser side effects [66] as opposed to already established models (such as the use of dimercaptosuccinic acid) which are often more expensive, not readily available, and burdened with undesirable side effects [65-67].

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

The objective of this review article is to find out the various metals induced toxicity on the male reproductive system especially on testicular system. The main aim is to collect the all data in a cumulative way and to compile all of these and represent them in a tabulated form.

MECHANISM OF TESTICULAR TOXICITY ON METAL EXPOSURE

Mechanism of testicular toxicity induced by dietary cadmium (Cd) has been less investigated than that following acute Cd injection. In the present study we characterized testicular injury in a small rodent, the bank vole, exposed sub-chronically to dietary Cd in a quantity of 0.9 μmol/g, and determined the importance of some factors (Cd accumulation, metallothionein (MT), oxidative stress, and zinc (Zn)) in the injury.

Dietary Cd induced moderate histopathological changes (hemorrhage in interstitium, necrosis and apoptosis in seminiferous tubule epithelium) in young (1 month old) bank voles fed, for 6 weeks, Fe-adequate (1.1–1.4 μmol/g) and Fe-enriched (4.5–4.8 μmol/g) diets. In contrast, adult (5 months old) bank voles appeared to be resistant to the toxic effects of dietary Cd, despite the fact that testicular Cd contents were higher and MT levels lower than those in the young animals. The Cd-induced histopathological changes and apoptosis were accompanied by increased testicular lipid peroxidation, decreased testicular Zn concentration and elevated levels of hepatic and renal MT and Zn. Supplemental dietary Zn (1.7–1.8 μmol/g) prevented the Cd-induced testicular Zn depletion and injury. The data indicate that dietary Cd produces testicular lesions indirectly, through decreasing testicular Zn, which seems to be due to the sequestration of this element by the Cd-induced hepatic and renal MT [68].

Table 1: Some examples of metal induced testicular toxicity with their toxic effects.

S. No.	Transition metal	Animal species/Strain	Route of exposure	Dose & Duration	Effect	Reference
1	**Cadmium (Cd)**	Human	Occupational (inhalation)	-	Testicular tumors and necrosis was observed.	[69]
		Mouse	Drinking water	30 ppm 7 weeks	Seminiferous tubules of testes showed complete absence of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa and loss of spermatogenesis.	[70]
		Culture of rat seminiferous tubules	-	0.1, 1 and 10 μg/L Over a 2-week	Dose-and-time-dependent alterations of the meiotic process of spermatogenesis. Increase of total abnormalities of fragmented sertoli cells.	[71]
2	**Cobalt chloride ($CoCl_2$)**	Hamster	-	I.p. 20, 10, 5 mg/kg b.wt. Single	Volume of seminiferous epithelium was significantly decreased whereas lumen diameter of seminiferous tubule and volume of interstitium was increased	[72]
		Mouse	Drinking water	200, 400, 800 ppm 12 week	Hypertrophy of interstitial Leydig cells, congested blood vessel and degeneration of spermatogonial cells and necrosis of both the seminiferous tubules and the interstitial tissue was observed	[73]
3	**Copper (Cu)**	Mouse	Drinking water	30 ppm 7 weeks	Seminiferous tubules showed complete absence of spermatogonia, spermatozoa and loss of spermatogenesis	[74]
4	**Manganese (Mn)**	Mouse	Oral	7.5, 15.0, and 30.0 mg/kg b.wt./Day, 43 days	Decrease in sperm motility and sperm counts, no alterations in the fertility or histology of the testes when compared with the controls.	[75]

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

5	**Molybdenum (Mo)**	Human Mice Mouse	Occupational Drinking water Oral	- ≥ 100 mg/L Sub acute 5, 10 or 20 mg/kg b.wt./day 35 days	Sperm abnormalities (broken flagellum, flagellum torso and separated flagellum) occur Adversely affects the sperm quality through inducing the testicular oxidative stress Epididymal sperm count and motility decreased and numbers of abnormal sperm were increased dose dependently	[76] [77] [78]
6	**Mercury (Hg)**	Human	Occupational	-	Inhibin B serum levels increased with increasing mercury exposure among the Greenland Inuit	[79]
7	**Mercury (Hg)**	Human	Diet (sea-food)	-	Infertile male with abnormal semen	[80]
8	**Mercuric chloride** **($HgCl_2$)**	Sprague-Dawley rats	Gavage	0.0, 1.0, or 2.0 mg/kg b.wt./day 60 days	Significant adverse effects on male rat reproduction endpoints including fertility	[81]
9	**Nickel chloride** **($NiCl_2$)**	Tropical fish (*G. carapo*)	-	I.p. 20 mg/kg b.wt. Single	Decrease in germinal epithelium and increase in the relative volume of the interstitium of seminiferous tubule. Diameter of the seminiferous tubule was markedly decreased	[82]
10	**Nickel (Ni)**	Mouse	-	Oral 5 and 10 mg/kg b.wt/day	35 days Decrease in sperm motility and sperm count was observed	[83]
11	**Nickel (Ni)**	Human (Indian welders) Occupational	-	-	A significant positive correlation between the percentage of tail defects in spermatozoa and blood nickel concentration was observed in workers	[84]

IN VITRO FERTILIZATION (IVF) AND SUBSEQUENT EMBRYONIC DEVELOPMENT PROCEDURE [85]

To investigate the effect of cadmium on fertilization and subsequent embryonic development, described in Chemicals section, stock solution of cadmium was used as a medium supplement. According to our preliminary experiments, we adopted three concentrations of 2.5 µg/ml, 5 µg/ml, and 10 µg/ml in the sperm exposure experiment both in mouse and human. Furthermore, we observed that cadmium exposure at a dose of 10 µg/ml for 30 minutes did not impair the motility of mouse spermatozoa. We placed mouse sperm, which were squeezed out of cauda epididymis, in cadmium-containing HTF-BSA for 30 min and then washed them with HTF-BSA before analyzing the motility parameters by CASA.

Next, the sperm were incubated in fresh HTF-BSA medium for another 60 min until capacitation was complete, after which a normal IVF procedure was performed. HTF-BSA medium was equilibrated in a 37°C, 5% CO_2 incubator one day before the experiment. A small volume of capacitated sperm suspension was added to a drop of 100 µl HTF-BSA medium containing freshly ovulated oocytes to achieve a final sperm concentration of 10^6/ml. Four to six hours later, fertilized oocytes at the pronuclear stage were washed and cultured in KSOM for *in vitro* development to the morula/blastocyst stages in 5% CO_2 in air. Oocytes were observed for male and female pronucleus formation (fertilization) at 6 h after the initiation of culture, and the numbers of 2-cell embryos, 4-cell embryos, morula and blastocysts after 24 h, 48 h, 72 h and 96 h in culture were checked and recorded, respectively.

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

IN VITRO CULTURE OF EMBRYOS DERIVED FROM NATURAL FERTILIZATION [85]

To study the post-fertilization effects of Cd on the development of embryos derived from natural fertilization, embryos were continuously exposed to cadmium from the zygote stage with 2 pronuclei produced by natural insemination to the blastocyst formation stage at exposure concentrations of 0.625 µg/ml or 1.25 µg/ml in KSOM culture medium *in vitro*. KSOM medium was equilibrated in a 37°C, 5% CO_2 incubator one day before the experiment. Zygotes were observed and recorded at the initiation of culture, and the number of 2-cell embryos, 4-cell embryos, morulae and blastocysts after 12 h, 36 h, 60 h and 84 h, respectively, were checked and recorded.

PREPARATION AND CULTURE OF SEMINIFEROUS TUBULES

The technique of seminiferous tubule culture has been described previously (Hue et al., 1998; Staub et al., 2000). Briefly, cultures (n = 3) were performed with and without fungicides. Stock solutions of CBZ, IPR and the mixture/cocktail (CCK) were prepared in 1% dimethyl sulfoxide (DMSO), and then diluted in serum-free culture medium to obtain the final concentrations of 500 nM or 50 nM in the basal compartment. The final medium and controls contained 0.03% DMSO. Basal media (with or without fungicides) were renewed every two days. For CCK, an equimolar solution of the two fungicides containing 0.03% DMSO (50 nM or 500 nM) was prepared [86].

IN VIVO STUDIES

Male reproductive toxicity of Nickel and putative protective role of melatonin have been assessed in 180 days old wistar rats. Ni was administered through drinking water and, melatonin (18:00 hrs) intraperitoneally for 60 days. On 61st day animals were sacrificed and testis was taken for estimation of tissue metal load by ICP-AES and biochemical estimation of antioxidants (both enzymatic- CAT, SOD, GPx, GR and non-enzymatic GSC, Ascorbic acid) together with level of lipid peroxidation. Serum titres of steroids and melatonin and activity of testicular steroidogenic enzymes (3β and 17β HSDH) were measured along with epididymal sperm parameters (Sperm count, motility and abnormality). Further, studies were extended to assess the effect of Ni on in vitro testosterone production by isolated rat Leydig cells under basal and stimulated conditions. Results of the present study reveal increased tissue metal content following Ni treatment. Ni induced oxidative stress in testis is marked by increased lipid peroxidation and depletion and inhibition of endogenous non-ezymatic and enzymatic antioxidants respectively. Marked inhibition of testicular steroidogenic enzymes was reflected in the form of altered serum titres of testosterone and estradiol in Ni administered animals. Disruption of spermatogenesis is marked by loss of sperms, decreased number of motile sperms and increased abnormal spermatozoa in the Ni treated group. Isolated Leyding cells incubated with Ni showed decreased testosterone secretion under both basal and hCG stimulated conditions. In conclusion, the present study has shown Ni induced oxidative stress and deleterious effects on steroidogenesis and adverse quantitative and qualitative effects on sperm. Melatonin has been found to be effective in preventing these effects to a greater extent though not completely [87].

In short, it is important to note that the endocrine disruption induced by Cd is likely to be multi-factorial, mediated via its effects on Leydig cells and/or the hypothalamic-pituitary-testicular axis. Therefore, one should critically analyze the in vivo effects induced by Cd regarding hormonal disruption, especially considering that the effects of Cd on the hormonal production and/or secretion, and if these effects are dose- and time-dependent. Moreover, the sequence of (or the concomitant) effects and the mechanisms involved remains to be explored and questions still remain unanswered. For example, it remains to be determined if Cd modulates AR and/or testosterone effects in the testis. Furthermore, the effects of Cd on the expression of androgen-regulated genes, both in vivo and in vitro, in the testis and/or Sertoli and germ cells will be helpful to address some of these questions [88].

SURVEY OF SOME LITERATURES:

Transition metals are *d* block elements, which show multiple oxidation states. Transition metals have a wide variety of applications in the industrial world. Recent studies have shown a considerable increase in metal contamination all over the world due to extensive use of metals and anthropogenic activity. A significant amount of many metals including transition metals have been reported in semen and blood of occupationally exposed workers. In the biological system, transition metals are mostly conjugated to proteins, forming metalloproteins, which are part of the enzymatic system. These are an essential component of biological function, but at higher concentration they can be toxic. Transition metals can interact additively, synergistically or antagonistically and affect each other's absorption, distribution and excretion. Recent studies have shown that exposure to transition metals such as Cd, Cr, Hg, manganese, Ni, V and Zinc affects many body organs including the reproductive system. Transition metals may adversely affect male reproductive system in the terms of disruption of spermatogenesis, reduction in sperm count, motility, viability and increase in oxidative stress, inhibition of testicular steroidogenesis, serum testosterone, libido and decline in fertility. Various underlined mechanism have been proposed for such effects. The aim of this review is to provide a summary of the effects of transition metal exposure on male reproductive organs and functions [89].

Isabel Corpas *et al*., reported that effects were reflected in the reduction of the thickness of epithelium and of seminiferous tubule diameter (STD) as a consequence of the action of lead in the reduction in numbers of prospermatogonia and spermatocytes. Atrophication of seminiferous tubules and the reduction in the number of Leydig cells in the Pb-treated group was also reported by Guang shan *et al.* Many studies previously by other researchers have also shown that reduction of germinal epithelium which was seems to be due to damage of germinal cells. Loss of germinal epithelium cells was also observed by Adhikari *et al*.. Moreover, the interstitial

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

space in adult testis was filled out with overgrowth of Leydig cells. This increase of Leydig and Blood cell was not caused by the reduction of the size of the seminiferous tubules but by enhanced mitotic activity of Leydig cells reported by Kissel *et al*., [90-95].

Arsenic is one of the most common toxins and a major health concern globally due to its wide distribution and adverse health effects. It is widely used as a feed additive in the production of animals at present. Arsenic has been proposed as a cause of reproductive failure in male workers at a copper smelter in Sweden, but this suggestion should be viewed with caution, as subjects were also exposed to other potentially hazardous metals (Beckman, 1978). Nevertheless, arsenic is considered to be a likely reproductive toxicant that might cause male-mediated reproductive effects (Hopenhayn-Rich et al., 1999). In the epididymides of mice, the presence of radioactive arsenic indicated a risk of decreasing sperm viability and impaired reproduction (Danielson et al., 1984; Pant et al., 2001). Furthermore, it was reported that arsenic exposure produced steroidogenic dysfunction causing impairment of spermatogenesis in rats (Sarkar et al., 2003) [96-101].

Effect of arsenic was studied on the testicular tissue of Swiss albino mice. Sodium-meta-arsenite ($NaAsO_2$) was administered to adult mice (25±30g) at a dose level of 30 mg/L and 40 mg/L through drinking water for 30, 45 and 60 days. After the treatment, the testicular organ was removed, weighed and processed for histopathological observation. No change in the body weight was recorded in treated groups after arsenic exposure but significant decrease in the relative testicular weight was observed in comparison with the control. The result showed that arsenictreated mice exhibited dose dependent gradual reductions in seminiferous tubular diameter and various gametogenic cell population i.e. resting spermatocyte, pachytene spermatocyte and step-7-spermatid except spermatogonia. Leydig cell atrophy was significantly increased in dose dependent manner indicating a definite effect of arsenic on the spermatogenesis in mice. These observations were supported by gradual reduction in Leydig cell population in the above treated groups. In conclusion, the above results confirm the toxic effect of arsenic in testis of mice [102].

The toxic effects of cadmium (Cd) on reproductive parameters are widely described in the literature. Experimental models often make use of the intraperitoneal route (i.p.), although human intoxication occurs preferentially by the oral route and can be continuous. However, little is known about the effect of Cd administration routes on the testicular structure. Thus, this study investigated the testicular impact of Cd exposure comparing both i.p. and oral routes, both single dose (SD), in addition to the oral route in fractional doses (FD). Swiss adult male mice received $CdCl_2$ 1.5 mg/kg i.p., 30 mg/kg oral SD, and 4.28 mg/kg oral FD for 7 consecutive days. The Cd bioaccumulation was observed in all routes, mainly in the oral FD route. The concentrations of testicular Ca and Cu decreased in all animals exposed to Cd, while Zn and Mn decreased only in the i.p. route. Testicular SOD activity was reduced in both routes of oral administration, while CAT increased in the i.p. route, and GST increased in all animals exposed to Cd. Changes in the tubular parameters and cell viability were observed in both routes of Cd administration but were more intense in the oral route, mainly in the FD. Serum testosterone concentration was reduced in both routes of oral administration. Tubular damage, such as the vacuolization of the seminiferous epithelium, germ cell detachment, and seminiferous tubule degeneration, occurred in all groups exposed to Cd. Therefore, the oral Cd administration presented greater potential to promote testicular damage, mainly when the metal was given in a fractionated way [103].

Cadmium (Cd) is an environmental toxicant and an endocrine disruptor in humans. Several organs (e.g., kidney, liver) are affected by Cd and recent studies have illustrated that the testis is exceedingly sensitive to Cd toxicity. More important, Cd and other toxicants, such as heavy metals (e.g., lead, mercury) and estrogenic-based compounds (e.g., bisphenols) may account for the recent declining fertility in men among developed countries by reducing sperm count and testis function. In this review, we critically discuss recent data in the field that have demonstrated the Cd-induced toxicity to the testis is probably the result of interactions of a complex network of causes. This is likely to involve the disruption of the blood-testis barrier (BTB) via specific signal transduction pathways and signaling molecules, such as p38 mitogen-activated protein kinase (MAPK). We also summarize current studies on factors that confer the testis sensitivity to Cd, such as Cd transporters and metallothioneins, and the impact of Cd on the testis as an endocrine disruptor, oxidative stress inducer and how it may disrupt the Zn+2 and/or Ca+2 mediated cellular events. While much work is needed before a unified mechanistic pathway of Cd-induced testicular toxicity is emerged, recent studies have helped to identify some of the likely mechanisms and/or events that take place during Cdinduced testis injury. Furthermore, some of the recent studies have shed lights on potential therapeutic or preventive approaches that can be developed in future studies by blocking or minimizing the destructive effects of Cd to testicular function in men [104].

Previously, they reported that Wistar–Imamichi (WI) rats are highly resistant to cadmium (Cd)-induced lethality and hepatotoxicity compared to Fischer 344 (F344) rats. Since the testes are one of the most sensitive organs to acute Cd toxicity, we examined possible strain-related differences in Cd-induced testicular toxicity between inbred WI and F344 rats. Rats were treated with a single dose of 0.5, 1.0 or 2.0 mg Cd/kg, as CdCl2, sc and killed 24 h later. Cd at doses of 1.0 and 2.0 mg/kg induced severe testicular hemorrhage, as assessed by pathological and testis hemoglobin content, in F344 rats, but not WI rats. After Cd treatment (2.0 mg/kg), the testicular Cd content was significantly lower in WI rats than in the F344 rats, indicating a toxiokinetic mechanism for the observed strain difference. Thus, the remarkable resistance to Cd-induced testicular toxicity in WI rats is associated, at least in part, with lower testicular accumulation of Cd. When zinc (Zn; 10 mg/kg, sc) was administered in combination with Cd (2.0 mg/kg) to F344 rats, the Cd-induced increase in

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

testicular hemoglobin content, indicative of hemorrhage, was significantly reduced. Similarly, the testicular Cd content was significantly decreased with Zn co-treatment compared to Cd treatment alone. Thus, it can be concluded that the testicular Cd accumulation partly competes with Zn transport systems and that these systems may play an important role in the strain-related differences in Cd-induced testicular toxicity between WI and F344 rats [105].

Cadmium (Cd) is a toxic heavy metal of both environmental and occupational concerns. The health impact of ethno-botanical approaches in attempts to ameliorate its deleterious effects in biological systems should be an area of scientific interest since established therapies are often burdened with undesirable side effects. Aim: To determine the effects of polyphenol-rich extract of the leaf of Vernonia amygdalina (PEVA) on Cd-induced testicular toxicity, oxidative stress, and histopathology in Wistar rats. Materials and Methods: A total of twenty five (25) male Wistar rats were divided into five groups as follows: Group 1 (Control) received distilled water (0.2 ml/100 g i.p.) for 5 consecutive days and thereafter left untreated for 28 days. Group 2 received Cd alone at 5 mg/kg (i.p.) for 5 consecutive days. Group 3 was pre-treated with Cd as Group 2 and thereafter left untreated for a period of 28 days, whereas Groups 4 and 5 were pre-treated with Cd as Group 2 and thereafter received PEVA (orally) at two dose levels (200 and 400 mg/kg, respectively) for 28 days. Results: Cd administration induced reproductive toxicity as evidenced by lowered level of follicle stimulating hormone, luteinizing hormone, and testosterone ($P < 0.05$); perturbation of sperm characterization ($P < 0.05$); deleterious disruptions of the antioxidant system as evidenced by lowered levels of reduced glutathione and superoxide dismutase as well as elevation in thiobarbituric acid reactive substances level ($P < 0.05$); decrease in relative testicular weight ($P < 0.05$); and severe disseminated necrosis of the seminiferous tubules with terminally undifferentiated/necrotic cells as revealed by the histopathological examination. These conditions were sustained following administration of the two dose levels of PEVA. Conclusion: PEVA administration is not a suitable therapeutic choice for fertility enhancement in male Wistar rat model of Cd-induced decline in reproductive function [106].

Cadmium is a heavy metal that is toxic to humans and the reproductive system. The present study aimed to investigate the mechanisms of cadmium-induced reproductive toxicity in a male Institute of Cancer Research mouse model of cadmium poisoning. Changes in luteinizing hormone receptor (LHR), 17α-hydroxylase and endothelial nitric oxide (NO) synthase (eNOS) expression levels were examined. A total of 24 male mice (4-week-old) were randomly divided into four groups (normal control group and low, medium and high cadmium groups) and subjected to gavage treatment with normal saline or cadmium-containing saline solutions for 8 weeks prior to sacrifice. To assess testicular injury, serum androgen levels were determined by ELISA, testicular tissue pathological changes were evaluated using hematoxylin and eosin staining. In addition, LHR, 17α-hydroxylase and eNOS expressions levels were examined by western blotting, and apoptosis was examined with a terminal deoxynucleotidyl transferase dUTP nick end labeling assay. The results demonstrated that the severity of testes injury increased with cadmium concentration. In addition, LHR, 17α-hydroxylase and eNOS expression levels increased with low and medium concentrations of cadmium; however, they were decreased following treatment with high concentrations of cadmium.

The results from the present study demonstrated that cadmium altered LHR, 17α-hydroxylase and eNOS expression levels in testicular stromal cells, which may impact testosterone synthesis. Furthermore, NO was suggested to be involved in cadmium-induced testicular injury by measurements of eNOS expression in testicular stromal cells [107].

Lead is an industrial pollutant that may exert specific toxic effect on mammals. The aim of study was to investigate the protective effect of vit. E and pumpkin seeds oil on male reproductive system of albino rat induced by lead toxicity , the studying toxic effect of lead on body weight , testis weight, Glutathione, Glutathione-s- transferase, lipid peroxide, testosterone, lead residue , sperm gram, Nrf2 gene expression and histopathological change in testes of rat. Sixty male albino rats randomly divided into 4 groups. Group I was given olive oil 3 times a week orally, group II was given 1.5 g/L lead acetate daily in drinking water, group III was given lead acetate plus 600 mg/kg/ bwt vit. E orally 3 times a week, while group IV was given lead acetate plus 288 mg/kg/bwt pumpkin seeds oil orally 3 time a week. The experiment was extended for 8 weeks. Our results revealed significant decrease in body weight, GSH, GST, testosterone hormone level and sperm viability, and significant increase in MDA, concentration of lead, sperm abnormality and expression of Nrf2 gene in group II without any significant changes in testis and epididymis weight. Moreover, the pathological changes in testes showed focal degeneration with loss of spermatogenic series in the seminiferous tubules. All above mentioned result were significantly improved in group III & IV. In conclusion, vitamin E and pumpkin seeds oil have a protective effect on the testicular damage induced by lead [108].

The aim of the study is to investigate the effects of sub-chronic poisoning with arsenic on the testes of chickens treated. Seventy-two 1-day-old chickens were randomly divided into 4 groups and provided food with different doses arsenic. The histological changes were examined. The mRNA levels of inflammatory factors, including transcription factor nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), cyclooxygenase- 2 (COX-2), prostaglandin E synthase (PTGEs), and heat shock proteins (Hsps), including Hsp70, Hsp90, Hsp60, Hsp40, and Hsp27 were assessed by quantitative real-time PCR in the testes of chickens. The protein expressions of iNOS, Hsp60, and Hsp70 were detected by western blot. Increased mRNA and protein levels of inflammatory factors and Hsps with

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

testicular damage showed that arsenic-induced testicular toxicity includes inflammatory and heat shock response in chickens, and that increased Hsps levels may play a protective role in inflammation damage induced by arsenic on the testes of chickens. This study aimed to compare Cd exposure by intraperitoneal (i.p.) and oral routes, evaluating the testicular subacute and subchronic effects. Adult male mice were separated into three groups subdivided according to the experimental period (7 and 42 days after Cd exposure: subacute and subchronic effects, respectively): one group received water and two groups received $CdCl_2$ (1.2 mg/kg i.p. and 24 mg/kg oral). The testicular concentration of essential minerals and Cd, activity of antioxidant enzymes and markers of oxidative stress, histology, and testicular histomorphometry were evaluated. The subacute effect of oral Cd showed reduced Fe concentration, while Ca and Cu increased in this route. The subchronic effect promoted decreasing in Mg in i.p. and oral routes, whereas Zn decreased only in the oral, and the Fe concentration did not change. SOD activity decreased in the oral subacute evaluation and in both pathways, i.p. and oral routes, in the subchronic evaluation, while GST activity increased, and MDA concentration decreased. Labeling of apoptotic cells was increased in the subacute and subchronic evaluation. Seminiferous epithelium degeneration, death of germ cells, and Leydig cell damages occurred in i.p. and oral routes. However, these damages were more intense in the oral route, mainly evaluating the subchronic effects. The results confirm that the severity of Cd-induced testicular injury depends on the pathway, as well as the duration of exposure [109].

Cadmium (Cd) is an environmental toxicant and an endocrine disruptor in humans and rodents. Several organs (e.g., kidney, liver) are affected by Cd and recent studies have illustrated that the testis is exceedingly sensitive to Cd toxicity. More important, Cd and other toxicants, such as heavy metals (e.g., lead, mercury) and estrogenic-based compounds (e.g., bisphenols) may account for the recent declining fertility in men among developed countries by reducing sperm count and testis function. In this review, we critically discuss recent data in the field that have demonstrated the Cd-induced toxicity to the testis is probably the result of interactions of a complex network of causes. This is likely to involve the disruption of the blood-testis barrier (BTB) via specific signal transduction pathways and signaling molecules, such as p38 mitogen-activated protein kinase (MAPK). We also summarize current studies on factors that confer and/or regulate the testis sensitivity to Cd, such as Cd transporters and metallothioneins, the impact of Cd on the testis as an endocrine disruptor and oxidative stress inducer, and how it may disrupt the $Zn^{(2+)}$ and/or $Ca^{(2+)}$ mediated cellular events. While much work is needed before a unified mechanistic pathway of Cd-induced testicular toxicity emerges, recent studies have helped to identify some of the likely mechanisms and/or events that take place during Cd-induced testis injury. Furthermore, some of the recent studies have shed lights on potential therapeutic or preventive approaches that can be developed in future studies by blocking or minimizing the destructive effects of Cd to testicular function in men [110].

Cadmium (Cd) is a toxic heavy metal of both environmental and occupational concerns. The health impact of ethno-botanical approaches in attempts to ameliorate its deleterious effects in biological systems should be an area of scientific interest since established therapies are often burdened with undesirable side effects. Aim: To determine the effects of polyphenol-rich extract of the leaf of Vernonia amygdalina (PEVA) on Cd-induced testicular toxicity, oxidative stress, and histopathology in Wistar rats. Materials and Methods: A total of twenty five (25) male Wistar rats were divided into five groups as follows: Group 1 (Control) received distilled water (0.2 ml/100 g i.p.) for 5 consecutive days and thereafter left untreated for 28 days. Group 2 received Cd alone at 5 mg/kg (i.p.) for 5 consecutive days. Group 3 was pre-treated with Cd as Group 2 and thereafter left untreated for a period of 28 days, whereas Groups 4 and 5 were pre-treated with Cd as Group 2 and thereafter received PEVA (orally) at two dose levels (200 and 400 mg/kg, respectively) for 28 days. Results: Cd administration induced reproductive toxicity as evidenced by lowered level of follicle stimulating hormone, luteinizing hormone, and testosterone ($P < 0.05$); perturbation of sperm characterization ($P < 0.05$); deleterious disruptions of the antioxidant system as evidenced by lowered levels of reduced glutathione and superoxide dismutase as well as elevation in thiobarbituric acid reactive substances level ($P < 0.05$); decrease in relative testicular weight ($P < 0.05$); and severe disseminated necrosis of the seminiferous tubules with terminally undifferentiated/necrotic cells as revealed by the histopathological examination. These conditions were sustained following administration of the two dose levels of PEVA. Conclusion: PEVA administration is not a suitable therapeutic choice for fertility enhancement in male Wistar rat model of Cd-induced decline in reproductive function [111].

Lead is a ubiquitous environmental and industrial pollutant that may have its toxic effects on the male. The present experimental animal study was designed to observe the changes in the testis due to excessive use of lead. Developmental effects were monitored in the male offspring of Swiss mice from Postnatal Day (PND) 1 till PND 21. Lead acetate reduced the number of pups per litter, percentage of live offspring, and pups weight. Testicular morphology was severely affected in mice exposed to Lead acetate. Therefore, exposure during gestation and lactation adversely affects the reproductive system of male mice. In present study lead toxicity induced histological alterations in the various components of the testis. It is observed in present investigation that lead acetate given in high dose (640mg/kg/days) caused decreased body weight as compared to control males during developing period of pups. The microscopic examination revealed that lead induced apparent damage and reduction in the number of seminiferous tubules and primordial germ cells. Oral exposure of lead acetate changed the arrangement and shape of spermatogonia cells and reduced the number of sertoli cells. It also diminished the development of Leydig cells. We can conclude from our findings that lead acetate adversely affects developing testis of Swiss mice [112].

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

Mercury has been recognized as an environmental pollutant that adversely affects male reproductive systems of animals. This study examined the effects of mercuric chloride on the antioxidant system and histopathological changes and also evaluated the ameliorating effects of sodium selenite and/or vitamin E in the rat testis tissues. Sexually mature male Wistar rats (weighing 300-320g and each group six animals) were given mercuric chloride (1mg/kg bw) and/or sodium selenite (0.25mg/kg bw)+vitamin E (100mg/kg) daily via gavage for 4weeks. In the present study, mercuric chloride exposure resulted in an increase in the TBARS level and a decrease in the SOD, CAT, GPx activities, with respect to the control. Further, light microscopic investigation revealed that mercury exposure induced histopathological alterations in the testis tissues. Supplementation of sodium selenite and/or vitamin E to mercury-induced groups declined lipid peroxidation, increased SOD, CAT, GPx activities. While some histopathological changes were detected in mercuric chloride treated group, milder histopathological changes were observed in animal co-treated with sodium selenite and/or vitamin E supplementation to mercuric chloride-treated rats. As a result, mercuric chloride induced testicular toxicity is reduced by sodium selenite and/or vitamin E, but not ameliorate completely [113].

All forms of mercury are considered poisonous. Methylmercury, one organic form, is highly toxic to many organs. The aim of the present study was to assess the effects of this form on the reproductive system in the rat. For this, 20 male rats were divided into two groups. One, which is considered as reference, received tap water. The second group received tap water containing methylmercury at the rate of 20 mg l^{-1} for 8 weeks. At the end of the experiment, blood samples were collected for the determination of total mercury and plasma testosterone. The left testes were used for the determination of total mercury and histological examination. Appropriate centrifugation was applied on right testes to extract interstitial and seminiferous tubular fluids. The epididymides were homogenised for the sperm count. Our results showed a dramatic fall in the plasma testosterone in the contaminated animals. The fall in plasmatic testosterone seems to be in relation with the decrease in the secretion of testosterone. In association with this, the concentration of testosterone in seminiferous tubules fluid dropped about 55% in the poisoned animals in comparison with the controls. Despite this, no decrease in the epididymal sperm count in contaminated rats was observed [114].

This investigation was set out to determine whether mercury at a very low dose (4 ppm) induces testicular damage on murine testis, and if so whether the toxic effects of mercury could be prevented by zinc. One of the following solutions was administered in the drinking water of Balb/C male mice: (1) 4 ppm $HgCl_2$; (2) 800 ppm $ZnCl_2$; (3) 4 ppm $HgCl_2$+800 ppm $znCl_2$; or (4) deionised water; for 12 weeks. At the expiration of the treatment period, animals were sacrificed, testes excised and weighed, and epididymal sperm number taken. The testes were processed for histological examination. Both zinc and mercury significantly ($P<0.05$) decreased the absolute and relative testicular weights, with mercury producing the highest reduction in weight. Mercury reduced significantly ($P<0.05$) the epididymal sperm number, while zinc and mercury/zinc produced statistically same effect with control on the sperm number. Histological study showed that mercury at the concentration employed produced remarkable degenerative lesions on the testes, as the zinc-treated group showed a normal morphology. Majority of the animals in the mercury/zinc treated group exhibited complete or partial protection as evidenced by the morphology of the somniferous tubules. Zinc prevents mercury-induced testicular damage in mouse. These findings highlight the risks exposure to inorganic mercury might pose to male reproduction of mice, and suggests possible therapy with zinc [115].

CONCLUSION

The overall result of this review offer a confirmation that certain transition metals such as As, Cd, Cr, Hg, Mn, Mo, Pb, Ni, V and Zn may adversely affect the male reproductive functions such as spermatogenesis, sperm quality, secretory functions of accessory glands, libido, fertility, serum testosterone level and antioxidant defense system. The testicular toxicity might be due to the oxidative stress in the testis on metal exposure. The degeneration of testicular tissue may also be responsible for decreased testis weight which may result in decreased sperm production and reduced testosterone level.

REFERENCES:

1. Carbone P, Giordano F, Nori F, Mantovani A and Taruscio D: The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: A population-based case control study in ruralsicily. Int. J. Androl. 2007; 30:3-13.
2. Chowdhury AR: Recent advances in heavy metals induced effect on male reproductive function-A retrospective Al Ameen J. Med. Sci. 2009; 2:37-42.
3. Turgut G, Abban S, Turgut and Take G: Effect of overdose zinc on mouse testis and its relation with sperm count and motility. Biol. Trace Elem. Res. 2003; 96:271-278.
4. Veado MARV, Arante IA, Oliveira AH, Almeida MRMG, Miguel RA, Severo MI and Cabaleiro HL: Metal pollution in the environment of minas gerais state-Brazil. Environ. Monit. Assess. 2006; 117:157-172.
5. Kutlubay R, Oguz EO, Can B, Guven MC, Sinik Z and Tuncay OL: Vitamin E protection from testicular damage caused by intraperitoneal aluminium. Int. J. Toxicol. 2007; 26:297-306.
6. Carlsen E, Giwercman A, Keiding N and Skakkebaek NE: Evidence for decreasing quality of semen during past 50 years. Br. Med. J. 1992; 305:609-613.
7. Jensen TK, Sobotka T, Hansen MA, Pedersen AT, Lutz W and Skakkebaek NE: Declining trends in conception rates in recent birth cohorts of native Danish women: A possible role of deteriorating male reproductive health. Int. J. Androl. 2008; 31:81-92.

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

8. Traina ME, Ade P, Siepi G, Urbani E and Petrelli MG: A review of the effect of pesticide formulations on male fertility. Int. J. Environ. Health Res. 1994; 4:38-47.

9. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al. European Association of Urology guidelines on Male Infertility: the 2012 update. Eur Urol 2012; 62:324-32.

10. Rani DS, Vanniarajan A, Gupta NJ, Chakravarty B, Singh L, Thangaraj K. A novel missense mutation C11994T in the mitochondrial ND4 gene as a cause of low sperm motility in the Indian subcontinent. Fertil steril 2006; 86:1783-5.

11. Hassani HH, Mohamed WM, Hasan HR, Majeed BJ, Khalf ZS. Heavy Metal Pollution and Men Infertility in Al-Falluja City. Baghdad Sci J 2016; 13:819-28.

12. Mendiola J, Moreno JM, Roca M, Vergara-Juárez N, Martínez-García MJ, García-Sánchez A, et al. Relationships between heavy metal concentrations in three different body fluids and male reproductive parameters: a pilot study. Environ Health 2011; 10:6.

13. Corpas I, Castillo M, Marquina D, Benito MJ. Lead intoxication in gestational and lactation periods alters the development of male reproductive organs. Ecotoxicol Environ Saf 2002;53:259-66.

14. Fahim MA, Tariq S, Adeghate E. Vitamin E modifies the ultrastructure of testis and epididymis in mice exposed to lead intoxication. Ann Anat 2013; 195: 272-77.

15. Anjum MR, Madhu P, Reddy KP, Reddy PS. The protective effects of zinc in lead-induced testicular and epididymal toxicity in Wistar rats. Toxicol Ind Health 2017; 33:265-76.

16. P. Gao, S. Liu, W. Ye *et al.,* "Assessment on the occupational exposure of urban public bus drivers to bioaccessible trace metals through resuspended fraction of settled bus dust," Science of The Total Environment, vol. 508, pp. 37–45, 2015.

17. M. P. Waalkes, "Cadmium carcinogenesis," Mutation Research, vol. 533, no. 1-2, pp. 107–120, 2003.

18. S. Satarug, S. H. Garrett, M. A. Sens, and D. A. Sens, "Cadmium, environmental exposure, and health outcomes," Environmental Health Perspectives, vol. 118, no. 2, pp. 182–190, 2010.

19. F. de Souza Predes, M. A. S. Diamante, and H. Dolder, "Testis response to low doses of cadmium in Wistar rats," International Journal of Experimental Pathology, vol. 91, no. 2, pp. 125–131, 2010.

20. H. Y. Jung, D. W. Seo, C. O. Hong, J. Y. Kim, S. Y. Yang, and K. W. Lee, "Nephroprotection of plantamajoside in rats treated with cadmium," Environmental Toxicology and Pharmacology, vol. 39, no. 1, pp. 125–136, 2015.

21. M. do Carmo Cupertino, R. D. Novaes, E. C. Santos et al., "Cadmium-induced testicular damage is associated with mineral imbalance, increased antioxidant enzymes activity and protein oxidation in rats," Life Sciences, vol. 175, pp. 23–30, 2017.

22. M. C. Cupertino, R. D. Novaes, E. C. Santos et al., "Differential Susceptibility of Germ and Leydig Cells to Cadmium- Mediated Toxicity: Impact on Testis Structure, Adiponectin Levels, and Steroidogenesis," Oxidative Medicine and Cellular Longevity, vol. 2017, Article ID 3405089, 11 pages, 2017.

23. L. Minutoli, A. Micali, A. Pisani et al., "Flavocoxid protects against cadmium-induced disruption of the blood-testis barrier and improves testicular damage and germ cell impairment in mice [corrected]," Toxicological Sciences, vol. 148, no. 1, pp. 311–329, 2015.

24. F. Squadrito, A. Micali, M. Rinaldi et al., "Polydeoxyribonucleotide, an adenosine-A2A receptor agonist, preserves blood testis barrier from cadmium-induced injury," Frontiers in Pharmacology, vol. 7, 2017.

25. M. W. Hentze, M. U. Muckenthaler, and N. C. Andrews, "Balancing acts: molecular control of mammalian iron metabolism," Cell, vol. 117, no. 3, pp. 285–297, 2004.

26. D. Y. Ryu, S. J. Lee, D. W. Park, B. S. Choi, C. D. Klaassen, and J. D. Park, "Dietary iron regulates intestinal cadmium absorption through iron transporters in rats," Toxicology Letters, vol. 152, no. 1, pp. 19–25, 2004.

27. A. Djuric, A. Begic, B. Gobeljic et al., "Oxidative stress, bioelements and androgen status in testes of rats subacutely exposed to cadmium," Food and Chemical Toxicology, vol. 86, pp. 25– 33, 2015.

28. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, et al: Hormones and endocrine disrupting chemicals: Low dose effects and nonmonotonic dose responses. Endocr Rev 33: 378-455, 2012.

29. Medina MF, Arrieta MC, Villafañe MN, Klyver SMR, Odstrcil IMA and González ME: Early signs of toxicity in testes and sperm of rats exposed to low cadmium doses. Toxicol Ind Health 33: 576-587, 2017.

30. Akinloye O, Arowojolu AO, Shittu OB and Anetor JI: Cadmium toxicity: A possible cause of male infertility in Nigeria. Reprod Biol 6: 17-30, 2006.

31. Luevano J and Damodaran C: A review of molecular events of cadmium induced carcinogenesis. J Environ Pathol Toxicol Oncol 33: 183-194, 2014.

32. Niknafs B, Salehnia M and Kamkar M: Induction and determination of apoptotic and necrotic cell death by cadmium chloride in testis tissue of mouse. J Reprod Infertil 16: 24-29, 2015.

33. De Angelis C, Galdiero M, Pivonello C, Salzano C, Gianfrilli D, Piscitelli P, Lenzi A, Colao A and Pivonello R: The environment and male reproduction: The effect of cadmium exposure on reproductive function and its implication in fertility. Reprod Toxicol 73: 105-127, 2017.

34. Marettoá E, Maretta M and Leqáth J: Toxic effects of cadmium on testis of birds and mammals: A review. Anim Reprod Sci 155: 1-10, 2015.

35. Verqilio CS, Moreira RV, Carvalho CE and Melo EJ: Evolution of cadmium effects in the testis and sperm of the tropical fish Gymnotus carapo. Tissue Cell 47: 132-139, 2015.

36. Yang Q, Li P, Wen Y, Li S, Chen J, Liu X, Wang L and Li X: Cadmium inhibits lysine acetylation and succinylation inducing testicular injury of mouse during development. Toxicol Lett 291: 112-120, 2018.

37. Ali I, Damdimopoulou P, Stenius U, Adamsson A, Mäkelä SI, Åkesson A, Berglund M, Håkansson H and

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

Halldin K: Cadmium induced effects on cellular signaling pathways in the liver of transgenic estrogen reporter mice. Toxicol Sci 127: 66-75, 2012.

38. Schneider SN, Liu Z, Wang B, Miller ML, Afton SE, Soleimani M and Nebert DW: Oral cadmium in mice carrying 5 versus 2 copies of the Slc39a8 gene: Comparison of uptake, distribution, metal content, and toxicity. Int J Toxicol 33: 14-20, 2014.
39. Hu H, Lu X, Cen X, Chen X, Li F and Zhong S: RNA Seq identifies key reproductive gene expression alterations in response to cadmium exposure. Biomed Res Int 2014: 529271, 2014.
40. Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. Mol Aspects Med 2005;26:235-44.
41. Mehri A, Marjan RF. Trace elements in human nutrition: A review. Int J Med Invest 2013;2:115-28.
42. Apgar J. Zinc and reproduction: An update. J Nutr Biochem 1992;3:266-78.
43. Egwurugwu JN, Ifedi CU, Uchefuna RC, Ezeokafor EN, Alagwu EA. Effects of zinc on male sex hormones and semen quality in rats. Niger J Physiol Sci 2013;28:17-22.
44. Li Y, Wu J, Zhou W, Gao E. Effects of manganese on routine semen quality parameters: Results from a population-based study in China. BMC Public Health 2012;12:919.
45. Keen CL, Zidenberg-Cherr S, Lo¨nnerdal B. Nutritional and toxicological aspects of manganese intake: An overview. In: Mertz W, Aternathy CO, Olin SS, editors. Risk Assessment of Essential Elements. Washington, DC: International Life Sciences Institute; 1994. p. 221-35.
46. Godatvar PR. Shukra: A clinical and experimental study on therole of Svarna Bhasma in normozoospermia. MD Thesis Submitted to Gujarat Ayurved University. Jamnagar: Gujarat Ayurved University; 1995.
47. Hamad AR, Al-Daghistani HI, Shquirat WD, Abdel-Dayem M, Al-Swaifi M. Trace element levels in seminal plasma which associated with sperm quality in fertile and infertile men. J Reprod Infertil 2014;5:14-22.
48. Yokoi K, Uthus EO, Nielsen FH. Nickel deficiency diminishes sperm quantity and movement in rats. Biol Trace Elem Res 2003;93:141-54.
49. Marzec-Wróblewska U, Kaminski P, Lakota P. Influence of chemical elements on mammalian spermatozoa. Folia Biol (Praha) 2012;58:7-15.
50. Oura C, Toshimori K. Ultrastructural studies on the fertilization ofmammalian gametes. Int Rev Cytol 1990;122:105-51.
51. Barltrop D, Khoo HE. The influence of nutritional factors on leadabsorption. Postgrad Med J 1975;51:795-800.
52. Nawal KA, Ula AK, Ban TS. Protective influence of zinc on reproductiveparameters in male rat treated with cadmium. Am J Med Med Sci2015;5:73-81.
53. Imafidon CE, Akomolafe RO, Sanusi AA, Ogundipe OJ, Olukiran OS, Ayowole OA. Polyphenol-rich extract of Vernonia amygdalina (Del.)leaves ameliorated cadmium-induced alterations in feeding patternand urine volume of male Wistar rats. J Intercult Ethnopharmacol2015;4:284-92.
54. IPCS, (International Programme on Chemical Safety). Cadmium -Environmental Aspects. Geneva: World Health Organization; 1992.p.135.
55. WHO, (World Health Organization). Preventing Disease throughHealthy Environments, Exposure to Cadmium: A Major Public Health Concern. Geneva, Switzerland: WHO; 2010.
56. Lewis GP, Coughlin LL, Jusko WJ, Hartz S. Contribution of cigarettesmoking to cadmium accumulation in man. Lancet 1972;1:291-2.
57. Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Healtheffects of cadmium exposure – a review of the literature and a riskestimate. Scand J Work Environ Health 1998;24 Suppl 1:1-51.
58. Bernard A. Renal dysfunction induced by cadmium: Biomarkers ofcritical effects. Biometals 2004;17:519-23.
59. Nordberg G, Nogawa K, Nordberg M, Friberg L. Cadmium. In:Handbook on Toxicology of Metals. New York: Academic Press;2007. p. 65-78.
60. Prozialeck WC, Edwards JR, Woods JM. The vascular endotheliumas a target of cadmium toxicity. Life Sci 2006;79:1493-506.
61. Goering PL, Waalkes MP, Klaasen CD. Toxicology of cadmium.Handbook of Experimental Pharmacology; Toxicology of Metals,Biochemical Effects. Vol. 115. New York: Springer-Verlag; 1994.p.189-214.
62. Klaassen CD, Liu J, Choudhuri S. Metallothionein: An intracellularprotein to protect against cadmium toxicity. Annu Rev PharmacolToxicol 1999;39:267-94.
63. Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. J Environ Pathol Toxicol Oncol 2001;20:77-88.
64. Nagma M, Nagma JA, Jamal S, Abdur R. Protective effect of rutinagainst cadmium induced hepatotoxicity in Swiss Albino Mice.J Pharmacol Toxicol 2012;7:150-7.
65. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metalions. Free Radic Biol Med 1995;18:321-36.
66. Buzzle-Side Effects of Polyphenols. August, 24; 2010. Available from:http://www.buzzle.com/articles/polyphenols-side-effects.html. [Last accessed on 2016 Mar 22].
67. Doaa El-Nager M, Badr AA. Effect of corn oil, flax seed oil and black seed oil on testicular damage induced by lead acetate in Albino Mice: A histological study. Pak J Zool 2013;45:1083-9.
68. Elżbieta Bonda, Tadeusz Włostowski & Alicja Krasowska, Testicular toxicity induced by dietary cadmium is associated with decreased testicular zinc and increased hepatic and renal metallothionein and zinc in the bank vole (Clethrionomys glareolus), Biometals, (2004), volume 17, pages615–624.
69. Ragan HA, Mast TJ. Cadmium inhalation and male reproductive toxicity. Rev Environ Contam Toxicol 1990;114:1-22.
70. Al-Attar AM. Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. Saudi J Biol Sci 2011;18:63-72.

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

71. Geoffroy-Siraudin C, Perrard MH, Ghalamoun-Slaimi R, Ali S, Chaspoul F, Lanteaume A, et al. Ex-vivo assessment of chronic toxicity of low levels of cadmium on testicular meiotic cells. Toxicol Appl Pharmacol 2012;262:238-46.

72. Lukac N, Massanyi P, Zakrzeweski M, Toman R, Cigankova V, Stawarz R. Cobalt induced alterations in hamster's testes in vivo. J Environ Sci Health Tox Hazard Subst Environ Eng 2007;42:389-92.

73. Elbetieha A, Al-Tthani AS, Al-Tthani RK, Darmani H, Owais W. Effect of chronic exposure to cobalt chloride on the fertility and testes in mice. J Appl Biol 2008;2:1-6.

74. Al-Attar AM. Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. Saudi J Biol Sci 2011;18:63-72.

75. Ponnapakkam TP, Bailey KS, Graves KA, Iszard MB. Assessment of male reproductive system in the CD-1 mice following oral manganese exposure. Reprod Toxicol 2003;17:547-51.

76. Slivkova J, Popelkova M, Massanyi P, Toporcerova S, Stawarz R, Formicki G, et al. Concentration of trace elements in human semen and relation to spermatozoa quality. J Environ Sci Health A Tox Hazard Subst Environ Eng 2009;44:370-5.

77. Zhai XW, Zhang YL, Qi Q, Bai Y, Chen XL, Jin LJ, et al. Effects of molybdenum on sperm quality and testis oxidative stress. Syst Biol Reprod Med 2013;59:251-5.

78. Pandey R, Srivastava SP. Spermatotoxic effects of nickel in mice. Bull Environ Contam Toxicol 2000;64:161-7.

79. Mocevic E, Specht IO, Marott JL, Giwercman A, Jönsson BA, Toft G, et al. Environmental mercury exposure, semen quality and reproductive hormones in Greenlandic Inuit and European men: A cross-sectional study. Asian J Androl 2013;15:97-104.

80. Choy CM, Lam CW, Cheung LT, Briton-Jones CM, Cheung LP, Haines CJ. Infertility, blood mercury concentrations and dietary seafood consumption: A case-control study. BJOG 2002;109:1121-5.

81. Heath JC, Abdelmageed Y, Braden TD, Goyal HO. The effects of chronic ingestion of mercuric chloride on fertility and testosterone levels in male Sprague Dawley rats. J Biomed Biotechnol 2012;2012:815186.

82. Massanyi P, Zemanova J, Makarevich A, Chrenek P, Cigánková V, Toman R, et al. Effect of nickel administration in vivo on the testicular structure in male mice. Acta Vet Brno 2007;76:223-9.

83. Pandey R, Kumar R, Singh SP, Saxena DK, Srivastava SP. Male reproductive effect of nickel sulphate in mice. Biometals 1999;12:339-46.

84. Danadevi K, Rozati R, Reddy PP, Grover P. Semen quality of Indian welders occupationally exposed to nickel and chromium. Reprod Toxicol 2003;17:451-6.

85. Zhao LL, Ru YF, Liu M, Tang JN, Zheng JF, Wu B, Gu YH, Shi HJ. Reproductive effects of cadmium on sperm function and early embryonic development in vitro. PLoS One. 2017 Nov 2;12(11):e0186727. doi: 10.1371/journal.pone.0186727. PMID: 29095856; PMCID: PMC5667747.

86. Cedric Pisani, Sebastien Voisin, Karim Arafah, Philippe Durand, Marie-Hélène Perrard, et al.. Ex Vivo Assessment of Testicular Toxicity Induced by Carbendazim and Iprodione, Alone or in a Mixture. Altex-alternatives to animal experimentation, 2016, 33 (4), pp.393–413. ff10.14573/altex.1601253ff. ffhal-01444080f

87. Shail K Chaube, In Vivo and In Vitro Studies on Nickel Induced Reproductive Toxicity and the Protective Role of Melatonin. Biology of Reproduction 83(1):114 · November 2010, DOI: 10.1093/biolreprod/83.s1.289

88. Lafuente A, Cano P, Esquifino A. Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? Biometals. 2003;16:243–250.

89. Milan Chandel, Gyan Chand Jain, Toxic eff ects of transition metals on male reproductive system: A review, J Environ Occup Sci, Oct-Dec 2014, Vol 3, Issue 4, 204-13.

90. Corpas I, Castillo M, Marquina D. And Benito MJ: Lead Intoxication in Gestational and Lactation Periods Alters the Development of Male Reproductive Organs. Ecotoxicology and Environmental Safety. 2001; 53:259-266.

91. Shan G, Tang T. And Zhang X: The Protective Effect of Ascorbic Acid and Thiamine Supplementation against Damage Caused by Lead in the Testes of Mice. J Huazhong Univ Sci Technol Med Sci. 2009; 29:68-72.

92. Batra N, Nehru B, Bansal MP: Influence of lead and zinc on rat male reproduction at biochemical and histopathological levels. Journal of Applied Toxicology. 2001; 21, 507–512.

93. Batra N, Nehru B and Bansal MP: Reproductive potential of male portan rats exposed to various levels of lead regard to Zinc status. Br. J. Nutr. 2004; 91, 1887-1891.

94. Adhikari N, Sinha N, Narayan R and Saxena DK: Lead- induced cell death in testes of young rats. J. Applied Toxicol. 2001; 21, 275-277.

95. Kissel H et al.: Point mutation in kit receptor tyrosine kinase reveals essential roles for kit signalling in spermatogenesis and oogenesis without affecting other kit responses. EMBO J. 2000; 19, 1312-1326.

96. Beckman, L. 1978. The Ronnskar smelter-occupational and environmental effects in and around a polluting industry in northern Sweden. Ambio. 7:226–231.

97. Hopenhayn-Rich, C., I. Hertz-Picciotto, S. Browning, C. Ferreccio, and C. Peralta. 1999. Reproductive and developmental effects associated with chronic arsenic exposure. Ars. Expos. Hlth. Eff. 3:151–164.

98. Danielsson, B. R., L. Dencker, A. Lindgreen, and H. Tjalve. 1984. Accumulation of toxic metals in male reproductive organs. Arch. Toxicol. Supp. 7: 177–180.

99. Pant, N., R. Kumar, R. C. Murthy, and S. P. Srivastava. 2001. Male reproductive effect of arsenic in mice. BioMetals. 14:113–117.

100. Sarkar, M., G. R. Chaudhuri, A. Chattopadhyay, and N. M. Biswas. 2003. Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. Asian J. Androl. 5:27–31.

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/

101. Xiao Sun, Siwen Li, Ying He, Hongjing Zhao, Yu Wang, Xiangwei Zeng, and Mingwei Xing, Physiology and reproduction, Arsenic-induced testicular toxicity in Gallus gallus: Expressions of inflammatory cytokines and heat shock proteins, 2017, Poultry Science 96:3399–3406, http://dx.doi.org/10.3382/ps/pex073

102. Sanghamitra Sarkar, Hazra J., Upadhyay S. N., Singh R. K. and Amal Roy Chowdhury, Arsenic induced toxicity on testicular tissue of mice, Indian J Physiol Pharmacol 2008; 52(1) 84–90

103. Viviane G. S. Mouro ,1 Ana L. P. Martins,1 Janaina Silva,1 Tatiana P. Menezes, Marcos L. M. Gomes,2 Juraci A. Oliveira,1 Fabiana C. S. A. Melo , and Sérgio L. P. Matta, Cadmium (Cd) is a nonessential metal that can contaminate, Subacute Testicular Toxicity to Cadmium Exposure, Intraperitoneally and Orally, Hindawi , Oxidative Medicine and Cellular Longevity, Volume 2019, Article ID 3429635, 14 pages, https://doi.org/10.1155/2019/3429635

104. Erica R. Siu, Dolores D. Mruk, Catarina S. Porto, and C. Yan Cheng, Cadmium-induced Testicular Injury, Toxicol Appl Pharmacol. 2009 August 1; 238(3): 240–249. doi:10.1016/j.taap.2009.01.028.

105. Hideaki Shimada, Rika Narumi, Masaaki Nagano, Akira Yasutake, Michael P. Waalkes, Yorishige Imamura, Strain difference of cadmium-induced testicular toxicity in inbred Wistar–Imamichi and Fischer 344 rats, Arch Toxicol. 2009 July ; 83(7): 647–652. doi:10.1007/s00204-009-0442-y.

106. Christian Eseigbe Imafidon, Olatoye Taiwo Risikat, Bamidele Funminiyi Samuel, Ojo Opeyemi Esther, Ademoye Kehinde Aderonke, Cadmium-Induced Testicular Toxicity, Oxidative Stress and Histopathology in Wistar Rats: Sustained Effects of Polyphenol-Rich Extract of Vernonia Amygdalina (Del.) Leaf, J Interdiscip Histopathol. 2016; 4(3): 54-62. doi: 10.5455/jihp.20160618041629

107. Yaping Ren, Wenhua Shao, Lijun Zuo, Wei Zhao, Haizhang Qin, Yingjie Hua, Dejie Lu, Chao Mi, Sien Zeng and Liao Zu, Mechanism of cadmium poisoning on testicular injury in mice, Oncology Letters, 18: 1035-1042, 2019

108. Amany Ramah, Ragab M. EL-shwarby, Nabila M. A., Elham A. El-shewey, The effect of lead toxicity on male albino rats reproduction with ameliorate by vitamin E and pumpkin seeds oil, Benha veterinary medical journal, vol. 28, no. 1:43-52, march 2015.

109. Viviane Gorete Silveira Mouro, Verônica Andrade Siman, Janaína da Silva , Fernanda Carolina Ribeiro Dias, Eduardo Medeiros Damasceno, Marli do Carmo Cupertino, Fabiana Cristina Silveira Alves de Melo, Sérgio Luis Pinto da Matta , Biol Trace Elem Res, Cadmium-Induced Testicular Toxicity in Mice: Subacute and Subchronic Route-Dependent Effects. 2020 Feb;193(2):466-482. doi: 10.1007/s12011-019-01731-5. Epub 2019 Apr 27.

110. Siu, R Erica & Mruk, Dolores & Porto, Catarina & Cheng, C. (2009). Cadmium-induced Testicular Injury. Toxicology and applied pharmacology. 238. 240-9. 10.1016/j.taap.2009.01.028.

111. Christian Eseigbe Imafidon, Olatoye Taiwo Risikat, Bamidele Funminiyi Samuel, Ojo Opeyemi Esther, Ademoye Kehinde Aderonke Cadmium-induced testicular toxicity, oxidative stress and histopathology in Wistar rats: Sustained effects of polyphenol-rich extract of Vernonia amygdalina (del.) leaf, Journal of Interdisciplinary Histopathology, 2016; 4(3): 54-62 www.scopmed.orgDOI: 10.5455/jihp.2016061804162954

112. U. Garu, R. Sharma and I. Barber, Effect of lead toxicity on developing testis of mice, IJPSR, 2011; Vol. 2(9): 2403-2407

113. Suna Kalender, Fatma Gokce Uzun, Filiz Demir, Meltem Uzunhisarcıklı, Ayse Aslanturk, Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E, Food Chem Toxicol, 2013 May;55:456-62. doi: 10.1016/j.fct.2013.01.024. Epub 2013 Jan 29.

114. Hatem Moussa, Lamia Hachfi, Mouni Trimeche, Mohamed Fadhel Najjar, Accumulation of mercury and its effects on testicular functions in rats intoxicated orally by methylmercury, Andrologia 43(1):23-7 · February 2011 with 105 Reads , DOI: 10.1111/j.1439-0272.2009.01003.x

115. Azarnia Mahnaz, Sanaie-Mehr A., Mercury induces testicular damage protected by zinc in mice, Iranian journal of biology, 2003, Volume 15 , Number 3, 16-24.

Doi: 10.2016-37245516/; DOI Link :: http://doi-ds.org/doilink/07.2020-84816251/