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Detailed mechanism of cadmium toxicity

Aditi Ghosh*, Omprakash, Poonam Devi, Sanjanna Boya, Deepak Chopra
Department of Animal Biochemistry, ICAR-National Diary Research Institute, Karnal, Haryana, India

Abstract
The innate ability to have children is called fertility. The fertility rate is known as the number of children born per couple, individual, or population. 8–12% of couples are thought to have infertility globally. A subset of 3-5% of these couples experiences unexplained infertility. Recent estimates place the number of infertile couples at 60–80 million per year worldwide, with 15–20 million of those individuals living in India alone. People are exposed to metal aerosols at work and in the environment with the highest cadmium, which builds up in the male reproductive system. The presence of Nectin-2, a junction found in ectoplasmic specialization (ES), which is responsible for the blood-testis barrier, is the cause of the accumulation of cadmium in the male reproductive system. The preceding claim that the testis is more vulnerable to cadmium is supported by data that basal ES is the target of cadmium. Erectile dysfunction, one of the major problems underlying male infertility, affects up to 10% of men. Due to its disruption of the hypothalamus-hypophysis testis axis (HHT), impairment of the androgen receptor's conformation, and methylation of numerous genes, cadmium has been classified as an endocrine disruptor. It inhibits the STAR gene, which is in charge of moving cholesterol from external to internal mitochondria, P450scc, which aids in the conversion of cholesterol to pregnenolone, and other genes. Fecal microorganisms, or gut bacteria, play a positive role in the colon rectum by encouraging the production of short-chain fatty acids, and free amino acids, and favoring the transfer of secondary bile acids from primary bile acids. Ben Eiseman performed the first fecal microbiota transplant to cure a Clostridium difficile illness. Fecal microbiota transplantation is a novel approach that involves introducing feces into the recipient's GI tract via a variety of delivery techniques, including colonoscopy, nasogastric, nasojejunal, and others. The therapeutic effects of FMT can be explained by a mechanism known as competitive niche exclusion. An excellent illustration of how FMT restores normal host metabolism and gut microbiota is the restoration of Firmicutes phylum bacteria and secondary bile acid metabolism.

Keywords: Infertility, cadmium toxicity, fecal microbiota transplantation, gut microbiota

Introduction

The harmful effects of heavy metals on male reproduction have been a hot topic in toxicology during this massive period of industrialization and urbanization. When consumed, heavy metals—a class of metals with a density greater than that of water—always result in toxicity in the body of the consumer. Heavy metals (> 5mg/kg) include substances like lead, mercury, arsenic, cadmium, and others. But in this paper, we will be focusing on cadmium heavy metal, as ingestion of cadmium has come on the rise in the past few years. Most of the reports have shown that males are exposed to heavy metals either occupationally or environmentally or both. Intake of contaminated food is a usual source of heavy metals. Contamination can occur for a variety of reasons, including improper handling of food, food processing, transit, etc. (Kidd *et al*., 2002). Even a few reports have surfaced indicating the presence of heavy metals like Cd and Pb in water supplies, foods like spinach and cabbage, cereals, and cosmetics, among other things. Moreover, meta-analyses have brought about the detrimental effects of environmental pollutants on male reproduction. It has been widely known by now that environmental pollutants like a small amount of Pb, Cd, Hg, etc, pave the way to the generation of ROS. Due to cadmium's inclusion in legumes, green leafy vegetables, pesticides, and insecticides, intake has recently grown. Small in size, it enters the body quickly and builds up in different organs depending on the susceptibility (Faroon *et al.,* 2012). Compared to non-smokers, smoking causes a 4-5 fold increase in cadmium levels. Spermatozoa are highly vulnerable to reactive oxygen species because it consists of large amounts of membrane polyunsaturated fatty acids (PUFA). As a result, it causes DNA damage, testicular apoptosis, disruption of seminiferous epithelium, and abnormal sperm function leading to male infertility (Pant *et al.,* 2003) [28]. Due to the lack of a detoxification mechanism in the human body, these toxins build up in the male reproductive system and reduce fertility (Wan *et al.,* 2003) Male infertility and poor-quality sperm have earned a place as global issues. Heavy metals can damage Sertoli and Leydig cells as well as hinder the creation of testosterone. It accomplishes this by meddling at the structural and gene levels. Androgen Binding Protein (ABP), cadherins, occludins, and other neuroendocrine processes are also extremely vulnerable to these heavy metals (Chung and Cheng, 2001) [3]. It is believed that 40–50% of a couple's infertility is caused by male factors. Low sperm count, slow sperm movement, and aberrant sperm morphology, which are referred to as oligospermia, asthenospermia, and teratozoospermia, respectively, are the three most important indicators of male factor infertility (Kilchevsky *et al.,* 2012) [22]. The ratio of Firmicutes to Bacteroides is largely thought to be important for preserving gut homeostasis and safeguarding the host from the invasion of any alien bacteria (Nelson *et al.,* 2010) [19]. Humans contain 100 trillion microbes, therefore this directly or indirectly controls every cell in the body. According to reports, the gut microbiota has a bidirectional effect on male reproduction since some of them express 17-HSD, 3-HSD, and other steroid-metabolizing enzymes (Devendran *et al.,* 2017) [6].

Researchers are developing novel treatments to lessen the adverse effects of medications used to reduce male metal toxicity.

Effect of Cadmium

Cadmium is harmful because of its propensity to imitate divalent cations like Ca^{+2} and Mg^{+2}, which interfere with physiological processes in the body. Cd can cause genetic instability, protein and DNA damage, and ultimately apoptosis at the cellular level. In connection with this, it generates a great deal of oxidative stress, which in turn causes the polyunsaturated fatty acids in the membrane to degrade, which opens the door to the disorder of membrane structure (Dixon *et al.*, 1998) [10]. When it comes to morphology, Cd causes the diameter and length of seminiferous tubules to decrease. When instances of apoptosis were found in germinal cells, the fact acquired confidence. Henceforth, Cadmium has been identified as an apoptosis inducer. Some other reports have also depicted the picture of ER expansion, mitochondrial dilation, and vacuolization after being exposed to cadmium.

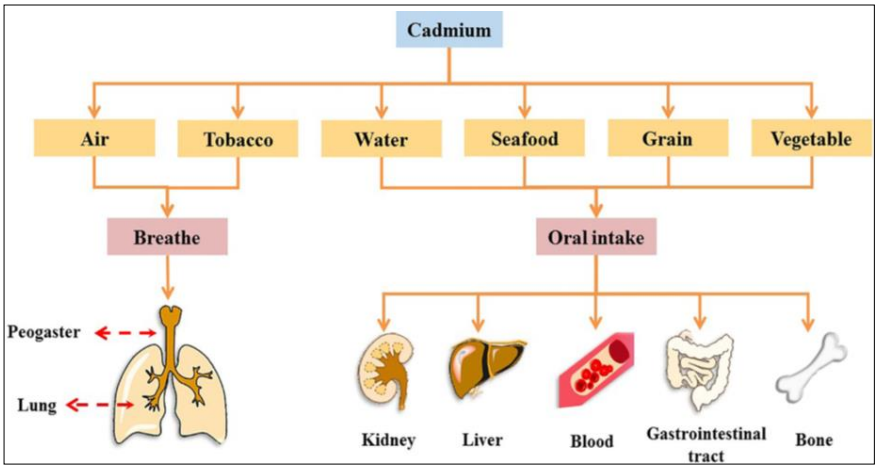

Fig 1: Major routes of Cd exposure and toxic effects of Cd on different organs in the human body (Wang *et al.*, 2021) [45]

Numerous reports have revealed various numerical values of half-lives in various body parts. For instance, the half-life in the kidney is 6-38 years, the liver is 4-9 years, and the blood is 75–128 days, but the corporal accumulation depends on the length and dosage of exposure because it mimics the critical divalent ions required for many physiological functions (WHO 2010). It is evident from the findings above that it has a significant impact on reproduction.

Mechanism of Cadmium toxicity

Utilizing the ability to imitate the divalent ions, Cadmium can interact with membrane transporters, allowing it to enter cells. Cadmium transport proteins are members of the Ca voltage-gated channel (VGCC) or Fe Transporters family of proteins. The digestive tract protein ZIP-8 and the divalent metal ion transporter DMT-1 have the highest Cd affinities, respectively (*Fujishiro et al.*, 2017) [16]. Male reproduction is one of the several processes that Cd mimics and interrupts. In particular, the absolute quantity of aspartate residues present at physiological pH has been demonstrated to be correlated with cadmium sensitivity and channel composition. It binds with albumin after being ingested, assisting with delivery to other organs. The liver, the body's first organ to which it gets there, later serves as the starting point for the production of metallothioneins (MT), a group of proteins with a high cysteine content that binds to metals (Fels *et al.*, 2019) [15]. Additionally, it has a strong propensity to cause oxidative

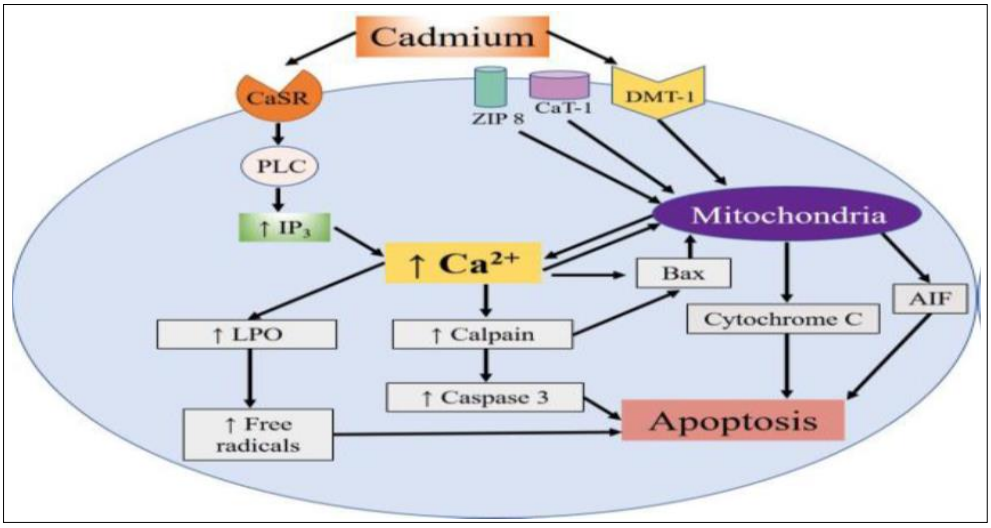

Fig 2: Apoptotic effects of cellular Cd reported in different cell types. CaSR: Ca- sensitive receptor, LPO: lipoperoxidation, DMT-1: Dimetallothionein, ZIP-8: Zinc transporter 8, AIF: Apoptosis-inducing factor (Arteaga *et al.*, 2021)

stress while subtly suppressing the action of the antioxidant enzyme. Additionally, it has extended its influence to mitochondria, where it degrades the -SH groups of cysteines found in the internal membrane proteins, uncoupling the energy that could have been retained for ATP generation. Additionally, it triggers the intrinsic process of apoptosis, which is signaled by the release of the proteins apoptosis-inducing factor (AIF) and mitochondrial cytochrome C. (Thevenod *et al.,* 2013). Additionally, it causes phospholipase C (PLC) to become activated, which raises the Ca concentration through an increase in the second inositol triphosphate messenger (IP3). By boosting the translocation of calpains (Ca-dependent cysteine proteases), these IP3 increases also cause the autolysis and activation of the pro-apoptotic protein Bax. It is further connected to the decline in GPX, CAT, and SOD activity as well as glutathione (GSH) levels. Even if Cadmium's principal target is mitochondria, it readily interacts with receptors in the plasma membrane by employing the ability to form multiple unions. This illustrates Cd's ability to modify signal transducing pathways. Another way of Cd toxicity includes alteration in the adhesion and migration since it can tune the structure of the cytoskeleton by modifying the concentrations and localizations of E-cadherins, N-cadherins, and β-catenin proteins (Prozialeck *et al.,* 2003) [31]. These proteins altogether are important components of tissue integrity. The mechanism behind modifying the structure is the mimicking power of the Cd.

Blood Testis Barrier: The major target of Cd

The blood-testis barrier is disrupted by heavy metals, most notably Cadmium, through certain signal-transduction pathways and particular proteins. Tight junctions (TJ), desmosome-like junctions (DLJ), gap junctions, and—most significantly—basal ectoplasmic specialization work together to form the blood-testis barrier. The basal ES is now an actin-based adherens junction that is exclusive to the testis. It should be highlighted that cohabitation is necessary to allow for periodic BTB reorganization to support stage VIII preleptotene spermatocyte migration. Due to its unusual morphological structure, it is susceptible to Cadmium. The principal target of the Cd is E-cadherin. Due to the calcium-binding motif on E-cadherin, Cd interacts with the motif and interferes with cell adhesion (Yan and Cheng, 2005) [48].

Signal transduction pathways control how harmful Cd is at the blood-testis barrier. When exposed to cadmium, the BTB produces more TGF-3, which in turn triggers the p38 MAPK signaling pathway. As a result, the levels of integral membrane proteins (such as occludin, N-cadherin, and E-cadherin) near the BTB location decrease. Additionally, proteases are activated, which further compromises the BTB's integrity and, as a result, results in the loss of germ cells from the seminiferous epithelium. This activates the c-JNK signaling pathway to inhibit proteolysis (Wong *et al.,* 2005) [35]. Few experiments have also reported the fact that

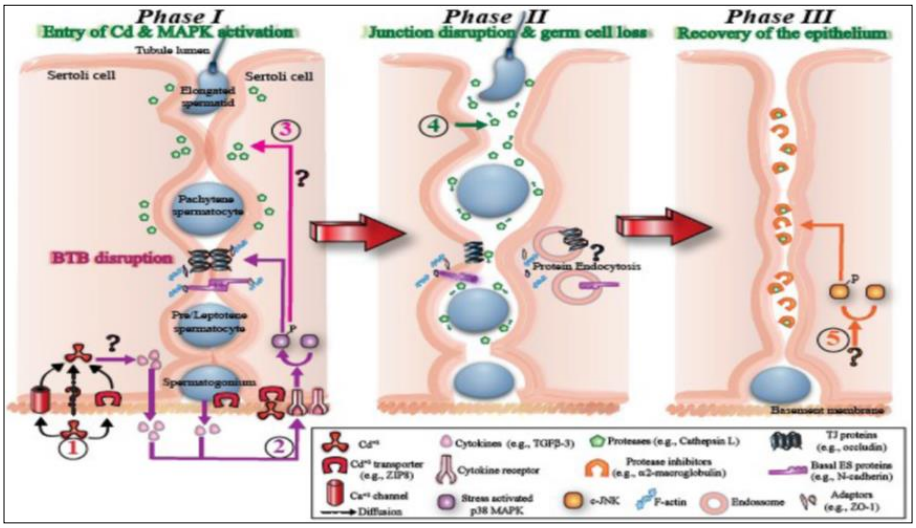

Fig 3: Cadmium-induced Blood –testis barrier (BTB) and cell junction disruption in seminiferous epithelium by activating specific signal transduction pathways. In phase 1 Cd enters the BTB barrier via Ca-gated channels or DMT in the Sertoli cells. After entering the Sertoli cells, it induces the synthesis of the cytokine TGF-β3, which interacts with their respective receptors and activates the stress-activated p38 MAPK signaling pathway. In phase II, BTB is disrupted via the degradation of integral membrane proteins as well as clathrin-mediated endocytosis of the tight junctions and basal ES proteins. In phase III, to limit the unwanted proteolysis, the c-JNK signaling pathway is activated which stimulates the release of proteases inhibitors (e.g α2-macroglobulin) to recover the seminiferous epithelium (Siu *et al.,* 2009) [34].

Nectin-2 which is a junction molecule present at the basal and apical ectoplasmic specializations (ES) for the formation of the Blood-testis barrier, is crucial for spermatogenesis. It has been established that Nectin-2 is what makes BTB more susceptible to infection than other tissues (Takai *et al.,* 2003) [40]. Since Nectin-2 is the flag protein for ES, it is thought that cadmium directly targets Nectin-2. According to the findings, Cd inhibits nectin-2 at the transcriptional and post-translational stages.

Table 1: The below table shows the effects of cadmium in testicular cells *in vivo*

***In vitro* model**	**Observation after exposure**	**References**
Primary rat Sertoli cell culture	Low-dose: impairment of development and maintenance of TJ-permeability barrier	(Janecki *et al.,* 1992) [20]

	High-dose: decrease in inhibin secretion and cell viability	(Janecki *et al.*, 1992) [20]
	Cell vacuolization and pyknosis; cell became round and then get detached from the substratum	(Syed *et al.*, 1997)
Rat Sertoli cell line cultures (ASC-D19)	TJs disruption, apoptosis	(Sorenson and Brabee, 2003)
Primary rat Sertoli cells and primary rat Leydig cell cultures	Sertoli cells are more sensitive to cadmium exposure than Leydig cells	(Clough *et al.*, 1990)
Primary rat Sertoli cells and gonocyte co-cultures	Morphological changes, decrease in cell viability, disruption of ubiquitin-dependent protein degradation	(Yu *et al.*, 2008)

The epididymis

The spermatozoids need to mature as well as leave the testis to fertilize an egg. By first passing via the epididymis, this maturation is accomplished. Three primary regions make up the epididymis: the caput, corpus, and cauda (Robaire *et al.*, 2015) [33]. Epididymis weight falls in response to exposure to cadmium, and the corpus shows the greatest deposition of Cd (Elgawish *et al.*, 2014) [12]. It is well-recognized that androgens are essential to the epididymis' ability to maintain a healthy epithelium. To ensure that proteins, glycoproteins, glycolipids, and phospholipids are properly secreted by the epididymis epithelium, which is necessary for spermatozoids to develop and survive, T secretion is crucial (Robbaire *et al.*, 2015). But when animals and the human gets exposed to Cd, it disrupts the androgen receptor, consequently decreasing their T concentrations and thereby reducing the size and weight of epididymis. Moreover, the epididymis is also responsible for conserving spermatozoid's genetic material while it transits to the fertilization site. Here, Cd causes the decompaction of chromatin by disrupting disulfide bridges, which makes spermatic DNA vulnerable to fragmentation (Olivia *et al.*, 2006). Cd lowers the concentration of N-acetyl glucosamine, sialic acid, and fucose in the membrane of spermatozoids which gives them the capacity to cross the cervical mucosa (Robaire *et al.*, 2015) [33]. However, when humans and animals are exposed to Cd, it affects the androgen receptor, which lowers their T levels and, as a result, decreases the size and weight of the epididymis. Additionally, the epididymis is in charge of protecting the genetic material of the spermatozoid while it travels to the site of fertilization. Here, Cd disrupts disulfide bridges to cause chromatin decompaction, leaving spermatic DNA prone to fragmentation (Olivia *et al.*, 2006). Cd makes spermatozoids' membranes less concentrated in N-acetyl glucosamine, sialic acid, and fucose, allowing them to pass the cervical mucosa (*Robaire et al.*, 2015) [33]. All of the aforementioned alterations can be directly attributed to the drop in androgens, which either directly or indirectly impedes all associated activities. Tyrosine phosphorylation is crucial for the development of spermatozoids' motility.

Cadmium as an endocrine disruptor

As a result of altering the steroidogenic area in Leydig cells, Cd controls the expression of the androgen receptor (AR) gene and reduces the ability of the body to produce testosterone. It decreases the amounts of steroidogenic acute regulatory protein (StAR), LH receptor, and cAMP in the testis (Henson *et al*., 2004) [18]. Additionally, it affects the hypothalamus-pituitary-testicular axis, which affects hormone levels. By attaching to each hormone's receptor, cd suppresses hormone production through competitive tactics. It can also compete with Dihydroxy testosterone (DHT), a naturally occurring ligand of AR. The transcription potential is decreased when Cd binds to AR and modifies its conformation (Lafuente *et al.*, 2003) [23]. Well, first of all, cAMP stimulates the production of T when cholesterol is transferred from the external to the internal mitochondrial membrane and kickstarts the production of pregnenolone with the aid of the enzyme P450scc. PKA is simultaneously activated by cAMP, and PKA in turn activates transcription factors that code for steroidogenic enzymes like StAR. Pregnenolone is now transformed into progesterone by the enzyme 3-hydroxysteroid dehydrogenase (3-HSD). 17-hydroxysteroid dehydrogenase (17-HSD) controls the conversion process, which results in androstenedione and eventually testosterone. Numerous investigations have demonstrated that Cd decreases the expression and activity of the 3-HSD and 17-HSD enzymes

Cadmium affects the immune system

The hypothalamus-hypophysis axis is damaged by Cd, which has long been known. Numerous cytokines are associated with the HHT axis to ensure its normal operation, including the proinflammatory cytokines tumor necrosis factor (TNF-), interleukin-1, and interleukin 6 (IL-6) (Diez-Pina *et al.*, 2009) [8]. TNF-, IL-1 and IL-6 levels significantly increase when the HHT axis is compromised. Additionally, it increases the levels of interleukin 10 (IL-10) and interferon-gamma (IFN), demonstrating Cd's capacity to increase inflammatory responses. Oxidative stress levels grow in tandem with the level of inflammation.

Multiple investigations have revealed elevated levels of TNF-, IL-1, IL-6, and IL-8, which were strongly linked to Cd-induced alteration of the gut flora. Clear photos of necrotic areas, short and thick villi, and many fusions were also displayed; these features were once more connected to an increase in the cytokines discussed earlier.

Cd has direct effects on gut microbiota!

Recent data suggest that exposure to heavy metals is the leading cause of the development of several metabolic illnesses, including type 2 diabetes, obesity, male infertility, and newborn infections. Heavy metals, especially Cadmium, are thought to be targets for the gut microbiota as a "superorganism." There are many HM-binding bacterial strains in the gut microbiota, including Lactobacillus, which is been shown to be beneficial for reducing heavy metal toxicity. Exposure to heavy metals and the gut microbiota composition are correlated in both directions since heavy metals can also hinder bacterial growth. By interfering with protein synthesis, cadmium limits the growth of bacteria. The majority of research has shown that Firmicutes and Proteobacteria abundance dropped after the following exposure, but the abundance of Bacteroidetes increased (Dheer *et al.*, 2015) [7]. All of these lead to an increase in fatty acid and lipid metabolism as well as increased

intestinal permeability. Additionally, Cd affects the gut flora in a metal-specific and time-dependent manner. In comparison to gram-negative bacteria, gram-positive bacteria are more susceptible as a result (Fazeli *et al.*, 2011) [14]. Similarly, Lactobacillus was less sensitive to Cd toxicity than Bifidobacteria, which may be due to Lactobacillus' considerable Cd-binding abilities (Bridou *et al.*, 2011) [2]. Therefore, it may be inferred from the aforementioned information that Cd can alter the composition of the gut microbiota, leading to an increase in LPS and inducing inflammation in the liver. By lowering the quantities of Akkermansia, which is in charge of both inducing TJ protein expression and maintaining the intestinal mucus layer, it can concurrently disrupt the gut barrier junctions. The overall increasing ratio of Bacteroidetes/Firmicutes and body fat is mostly due to these changes. The fact that subsequent antibiotic treatment effectively halted the buildup thus far after fecal microbiota transplantation from Cd-exposed animals to control mice was performed, suggests that gut microbiota play a significant role in the entire process.

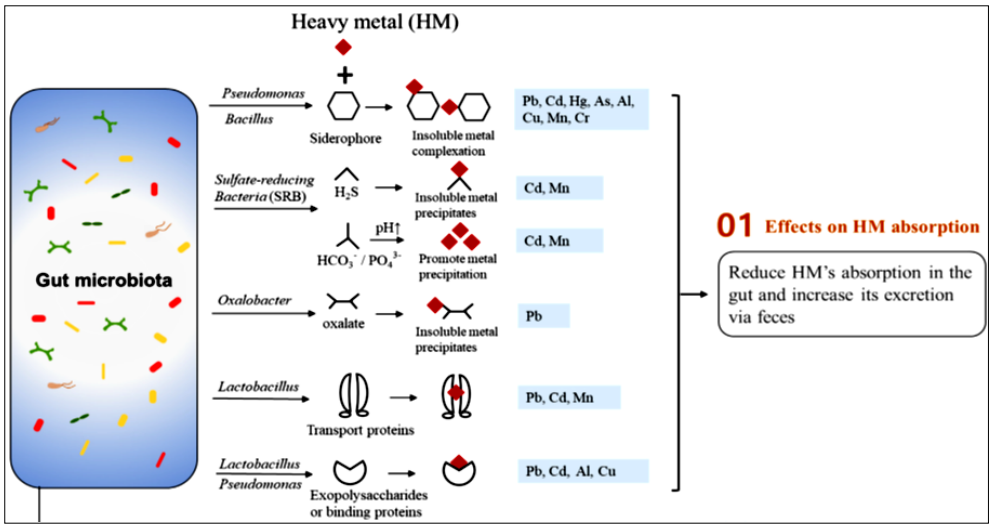

Fig 4: Direct effects of gut microbiota on the absorption of heavy metals (Duan *et al.*, 2020) [11]

The interplay between Androgen and gut microbiota

The primary active forms of androgen, testosterone (T) and dihydrotestosterone (DHT), are the cornerstones for the development and maintenance of masculinization, normal sexual function, and spermatogenesis. Androgen has always served as a link between the endocrine system and the reproductive system (Matsumoto *et al.*, 2013) [25]. Nearly 37.8% of senior men have testosterone insufficiency, also known as hypogonadism, which causes obesity, decreased glucose tolerance, metabolic syndrome, osteoporosis, etc (Mulligan *et al.*, 2006) [26]. As the title suggests, multiple processes allow testosterone and gut microbiota to alter one another. Most of the bacteria are found in the gut, and it is known that the colon and rectum are where the androgen receptor is specifically expressed. Therefore, the gut microbes are constantly close to the androgen receptor. It is well established that androgen regulates stromal cell BMP and Wnt/TGF- signaling, maintaining intestinal homeostasis. As a result, androgen regulates the intestinal barrier and microenvironment, changing the gut flora. Additionally, it should be mentioned that the gut microbiota varies depending on gender. Prevotella is intimately correlated with testosterone synthesis, therefore men have higher levels of it. Bile aids in the direct excretion of glucuronidated androgens into the small intestine. Degluronidated testosterone (T-G) and dihydrotestosterone (DHT-G) are produced by the glucuronidase-dependent gut microbiota in the cecum before being absorbed in the distal intestine. Additionally, this testosterone production encourages the development of microbes. Additionally, the Clostridium and Ruminococcus species have a remarkable capacity to transform the androgens glucocorticoids, pregnenolone, and hydroxypregnenolone (J.M. *et al.*, 2013). In conclusion, it has been discovered that gut microbiota including Butyricoccus desmolans, Clostridium cadaveris, and Clostridium scindens express steroid processing enzymes like 17-Hydroxysteroid (17-HSD),3-HSD. As a result, levels of T metabolites, including DHT, and 3-diol concentrations, are found to be higher in the colon than in plasma (Devendran *et al.*, 2017) [6].

Several Fecal Microbiota Transplantation (FMT) studies have been conducted and showed that different gut microbiota can either enhance or disrupt the process of spermatogenesis. When FMT from High-fat diet (HFD) mice to normal diet mice were done, it disrupted the spermatogenesis process.

Fecal Microbiota Transplantation

To reconstruct gut dysbiosis, a treatment known as fecal microbiota transplantation involves administering a fecal matter solution that has been collected from a healthy donor animal into the recipient's intestinal tract. FMT involves several major phases, including choosing the donor animal, gathering the feces, processing them, preparing the recipient, and then transferring the feces to the recipient (Smits *et al.*, 2013) [36]. There are several ways to administer feces including colonoscopy, nasogastric tube, and nasojejunal tube (Postigo *et al.*, 2012) [30]. When it successfully treated Clostridium difficile illness, FMT gained notoriety (CDI). By inhibiting the pathogenic microbiota competitively, FMT restores the normal gut flora (Choi *et al.*, 2016). It inhibits the pathogenic microbiota through the production of secondary bile acids from primary bile acids, boosting the production of free amino acids, and increasing the production of SCFA thereby lowering the pH. (Stellwag and Hylemon,1978) [38]. As has already been said

in this book chapter, heavy metals modify the gut microbiota, and vice versa, the gut microbiota also has an impact on the absorption of heavy metals. The abundance of beneficial bacteria in the gut, including Bifidobacteria, Lactobacillus, Butyricoccus, etc., is decreased by heavy metal toxicity. Therefore, when feces from a healthy donor are given to the patient, the quantity of beneficial microbes quickly increases, ultimately improving the gut barrier, immunity, and SCFA synthesis. Additionally, these microbes will bind with heavy metal and produce toxic heavy metal precipitates, aiding in its elimination. Even yet, more investigation is needed to clarify the mechanism through which FMT reduces the toxicity of heavy metals.

Conclusion

Exposure to heavy metals has significantly increased as a result of excessive industrialization. Heavy metals are those metals that are hazardous to humans and have a higher density. A few examples of heavy metals are cadmium, lead, arsenic, and mercury. Because most herbicides, insecticides, batteries, automotive exhaust, etc. contain cadmium, which people are exposed to daily and eventually accumulate in the body for many years, cadmium has now become the element that poses the greatest risk to humans. Males are exposed to cadmium either through their environment or at work, which damages their reproductive system and causes asthenospermia, oligospermia, and azoospermia. It has been established that the blood-testis barrier is Cadmium's main target This is the case because BTB contains the cadmium-susceptible nectin protein and basal ES. Additionally, it inhibits the STAR gene, the Luteinizing Hormone Receptor (LHR), and the Androgen Receptor (AR), which are essential for the efficient operation of spermatogenesis. In this situation, the gut microbiota is important because heavy metals and gut bacteria interact. Metals can be chelated by microbiota like Lactobacillus and Pseudomonas, particularly Cd, which is then precipitated in the path. The system is protected by the gut microbiota in various ways, including by reducing pH, upping SCFA synthesis, boosting immunological function, and many others. Fecal microbiota transplantation has therefore emerged in the middle as the answer to lowering heavy metal toxicity, particularly cadmium. Fecal matter contains millions of gut microbes that, when properly fed into the colon, can outperform harmful bacteria in several ways, boosting the immune system and reinforcing the gut barrier. The FMT effect on heavy metal toxicity needs to be confirmed by more studies without a doubt.

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