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# Mechanisms of diazinon effects on impaired spermatogenesis and male infertility

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## Abstract

Diazinon (DZN) is an organophosphate insecticide that has cytotoxic and pathological effects on the reproductive system. It causes a wide variety of pathological effects on the reproductive system such as testicular atrophy, disturbance in sex hormones, impaired spermatogenesis, low quality of sperm, and fertility problems. However, molecular and cellular mechanisms of its adverse effects are not well understood. General events such as testicular damage, inflammation, mitochondrial deficiency, DNA fragmentation, disintegration of sperm plasma membrane, apoptosis, and cell death are observed in DZN-exposed animals. Oxidative stress (OS) induced by reactive oxygen species may be a main mechanism, which can be associated with sperm DNA fragmentation, reduced integrity of sperm cell membrane, apoptosis, depletion of antioxidants, and subsequently poor sperm quality and male infertility. Therefore, identification of these pathways may provide valuable information regarding the mechanisms of DZN action on the male reproductive system. In this review, we aim to discuss the proposed cellular and molecular mechanisms of DZN action on male reproductive system, the importance of OS and mechanisms by which DZN induces OS and depletion of other antioxidants.

## Keywords

Diazinon, oxidative stress, reproductive system, sperm, male infertility

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## Introduction

Environmental contamination with pesticides, especially organophosphate (OPs) insecticides, is a major health problem throughout the world. OPs constitute 70% of the insecticides used in the United States (Oostingh et al., 2009). Diazinon (DZN) is one of the OPs insecticides that is widely used in agriculture for crop protection and pest control (Jorsaraei et al., 2010). DZN exposure can be associated with severe health problems in humans and other mammals. Upon absorption from the gastrointestinal tract, DZN inhibits the activity of acetyl cholinesterase, resulting in an accumulation of acetyl choline, affecting neuromuscular transmission (Boussabbeh et al., 2016; Kalender et al., 2005; Perry et al., 2011).

The toxicity of DZN has been widely studied in animal models, but the human data are very limited. Nevertheless, a growing number of studies have indicated that intensive use of DZN can be also dangerous for humans. Occupational exposure to higher

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**Table 1.** Biomarkers of DZN action on different cell types.

| Study model | Results | References |
|---|---|---|
| *In vivo* | | |
| Rat testis | ↑MDA; ↓GSH; ↓vitamin C; ↓vitamin E; ↓$\beta$-carotene | Oksay et al. (2013) |
| Rat testis | ↑MDA; ↓GSH | Rahimi Anbarkeh et al. (2014) |
| Rat liver | ↑MDA; ↑caspases-9; ↑caspases-3; ↑Bax/Bcl-2 ratio | Lari et al. (2015) |
| Rat liver | ↓CAT activity; ↓peroxiredoxin-6 activity; ↓3-ketoacyl-CoA thiolase activity | Lari et al. (2014) |
| Rat liver | ↓SOD activity; ↓CAT activity; ↓vitamin C | Ahmadi-Naji et al. (2017) |
| Rat serum | ↑TNF-$\alpha$; ↑8-iso-prostaglandin F2$\alpha$ | Moallem et al. (2014) |
| Rat serum | ↑ALT; ↑AST; ↑NO; ↑MPO; ↓GPX; ↓SOD | Beydilli et al. (2015) |
| Rat serum | ↑ALT; ↑AST; ↑ALP; ↑VLDL; ↑Cho | Kalender et al. (2005) |
| Rat serum | ↑MDA; ↑PC; ↑TNF-$\alpha$; ↓HDL; ↑VLDL; ↑TC | Ahmadi-Naji et al. (2017) |
| Rat serum | ↑Urea; ↑creatinine; ↑MDA; ↑urinary glucose | Boroushaki et al. (2013) |
| Rat serum | ↑COX-2; ↑iNOS; ↑IL-6; ↑TNF-$\alpha$ | Ogasawara et al. (2017) |
| Mice serum | ↑LDL; ↑VLDL; ↑Cho; ↑creatinine; ↓glucose; ↓RBC; ↓Hb; ↓HCT; ↓PLT; ↑lymphocytes, ↑neutrophils | Zeinali et al. (2017) |
| Rat pancreas | ↓Viability of cells; ↑blood glucose; ↓weight; ↑OS markers; ↑caspases-9; ↑caspases-3; ↑ATP depletion | Khaksar et al. (2017) |
| Rat heart | ↑MDA; ↓GSH; ↑caspase-3; ↑Bax; ↓Bcl-2; ↑cytochrome C; ↑Bax/Bcl-2 ratio | Razavi et al. (2013) |
| *In vitro* | | |
| HCT116 cell lines | ↑ROS; ↑MDA; ↑DNA fragmentation | Boussabbeh et al. (2016) |
| PaTu cell line | ↑Caspases-3, -9; ↓thiol molecules; ↓mitochondrial activity; ↓mitochondrial membrane potential | Shiri et al. (2016) |
| Human sperm | ↓Sperm quality; ↑DNA fragmentation | Salazar-Arredondo et al. (2008) |

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Bcl-2: B-cell lymphoma 2; CAT: catalase; COX: cyclooxygenase; DZN: diazinon; GPX: glutathione peroxidase; GSH: glutathione; HCT: hematocrit; HDL: High-density lipoproteins; iNOS: inducible nitric oxide synthase; IL-6: Interleukin 6; LDL: Low-density lipoprotein; MDA: malondialdehyde; MPO: Myeloperoxidase; NO: Nitric oxide; OS: oxidative stress; PC: protein carbonyl; PLT: platelets; ROS: reactive oxygen species; SOD: superoxide dismutase; TC: total cholesterol; TNF-$\alpha$: tumor necrosis factor-$\alpha$; VLDL: Very-low-density lipoprotein.

concentrations of DZN in the spraying season may lead to side effects in different organs (Lu et al., 2006).

Although DZN affects mainly the nervous system, it can lead to some complications in various organs and systems (Table 1). General signs and symptoms include headache, dizziness, weakness, blurred vision, nausea and vomiting, as well as abdominal cramps, diarrhea, pinpoint pupils, difficulty breathing, coma, and possibly death (Toman et al., 2009). Some studies reported hyperglycemic and hemostatic disorders related to glucose metabolism in DZN-exposed individuals. For example, in a more recent study, Khaksar et al. (2017) have demonstrated increased blood glucose levels and significant weight loss after DZN exposure. The genotoxic potential of DZN and its cytotoxic effect on human peripheral lymphocytes was also reported (Muranli et al., 2015). Tisch et al. (2002) showed the potential carcinogenicity of DZN to human nasal mucosal cells. Toxicity effects of DZN on liver, kidney, and brain were also previously reported in animal models (Ezzi et al., 2016; Tsitsimpikou et al., 2013).

Recent experimental studies have indicated that the reproductive system is a main target for DZN toxicity. However, the effect of DZN on human male infertility is still controversial. Some *in vivo* studies demonstrated the pathological effects of DZN on reproductive function with decreased levels of libido and androgenic hormone (Maxwell and Dutta, 2005). A great number of experimental studies have also revealed that DZN can lead to male reproductive toxicity through spermatogenesis deficiency, a decline in androgen levels, abnormal sperm, and a direct cell

killing action (Rahimi Anbarkeh et al., 2014). However, cellular and molecular mechanisms by which DZN affects spermatozoa and induces poor sperm quality are still not well-understood. Therefore, there is a need for further detailed studies with a focus on the underlying mechanisms by which DZN induces reproductive dysfunction and male infertility. In the following sections, we will discuss general reproductive effects of DZN, as well as possible mechanisms by which it affects spermatogenesis and male infertility.

## Materials and methods

The articles discussed in this review were obtained by searching PubMed, Science Direct, Scopus, Google Scholar, and ISI Web of Knowledge. We searched articles that were published from 1970 to 2017. To identify relevant articles, we used the following keywords: "diazinon," "organophosphorus," "fertility," "male infertility," "spermatogenesis," "testes," "testicular damages," "sperm," "semen," "spermatozoa," "oxidative stress," "apoptosis," "DNA damage," "reactive oxygen species," "sex hormones," "endocrine," and "mitochondrial deficiency." Initial search yielded a total of 384 articles that were collected, read, and classified as relevant or irrelevant for the literature review. Relevant articles, at least to some degree, had to examine the relationship between DZN with impaired spermatogenesis and infertility. Ultimately, a total of 94 articles were found that met this criterion. The articles included original animal, *in vitro*, and human studies. All of these articles were published as peer-reviewed journals.

## Structural changes

A large number of experimental studies have shown that DZN has a significant effect on testes structure and function (Adamkovicova et al., 2014; Dutta and Meijer, 2003; Jorsaraei et al., 2010). Jorsaraei et al. (2010) found that intraperitoneal DZN administration (25 mg/kg) causes a significant reduction in both seminiferous tubule size and germ cells count. The intraperitoneal and oral administration of DZN has been also reported to be associated with degeneration and necrosis of seminiferous epithelium, tubule deluminization, and testicular atrophy (Toman et al., 2009). These lesions can lead to decreased fertility or induced infertility (Toman et al., 2016). Dutta and Meijer (2003) indicated a significant reduction in lumen diameter of tubules, seminiferous tubule diameter, number of germ cells, and spermatozoa after 2 weeks of exposure to DZN. Reduced number of spermatogenic, Sertoli, and Leydig cells were found following exposure to DZN (Hatjian et al., 2000; Maitra and Sarkar, 1996; Salem, 1998). Toman et al. (2009) found that germ cells lost their contact with the basal lamina after DZN treatment. Another histological study showed disruption and sloughing of basal germinal epithelium and vacuoles after oral administration of DZN in rat testis (Damodar et al., 2012). Since seminiferous tubules are a main source of sperm production, adverse effects of DZN on their structure can be associated with impaired spermatogenesis and reduced number of spermatozoa.

Several studies have also reported that DZN can cause a decrease in reproductive organ weights, such as seminal vesicle and prostate (Abd el-Aziz et al., 1994; Jayachandra and D'Souza, 2013). El-Hoda and Zidan (2009) observed a reduction in the testis and vesicular gland weights after intraperitoneal treatment to DZN. Testicular atrophy with weight loss was observed after DZN treatment (10 mg/kg) in male dogs (Earl et al., 1971). Therefore, degenerative changes in testicular structure can be considered as one of the main mechanisms of DZN that may be associated with impaired spermatogenesis, decrease in the number of spermatocytes, spermatids, spermatozoa, poor sperm quality, and eventually fertility failure (Figure 1).

## Spermatogenesis deficiency

Increaseing evidence has demonstrated that DZN can lead to fertility problems through the inhibition of spermatogenesis (Dutta and Meijer, 2003; Pina-Guzman et al., 2005). Earl et al. (1971) showed testicular atrophy with completely arrested spermatogenesis after DZN treatment in male dogs. Similarly, Fattahy et al. (2007) showed that DZN can arrest spermatogenesis, which is subsequently associated with reduced number of germ cells, blood vessels, spermatocytes, spermatids, and sperm cells. Several studies have also shown reduced numbers of Leydig and Sertoli cells after DZN treatments, which is subsequently associated with decreased level of serum testosterone and impaired spermatogenesis (Fattahi et al., 2009) (Figure 1). Therefore, DZN can cause impaired spermatogenesis through the induction of testicular cell damage and reduced level of testosterone. However, oxidative stress (OS) induced by reactive oxygen species (ROS) is the other significant mechanism of DZN toxicity that can be associated with germ cell damage and impaired

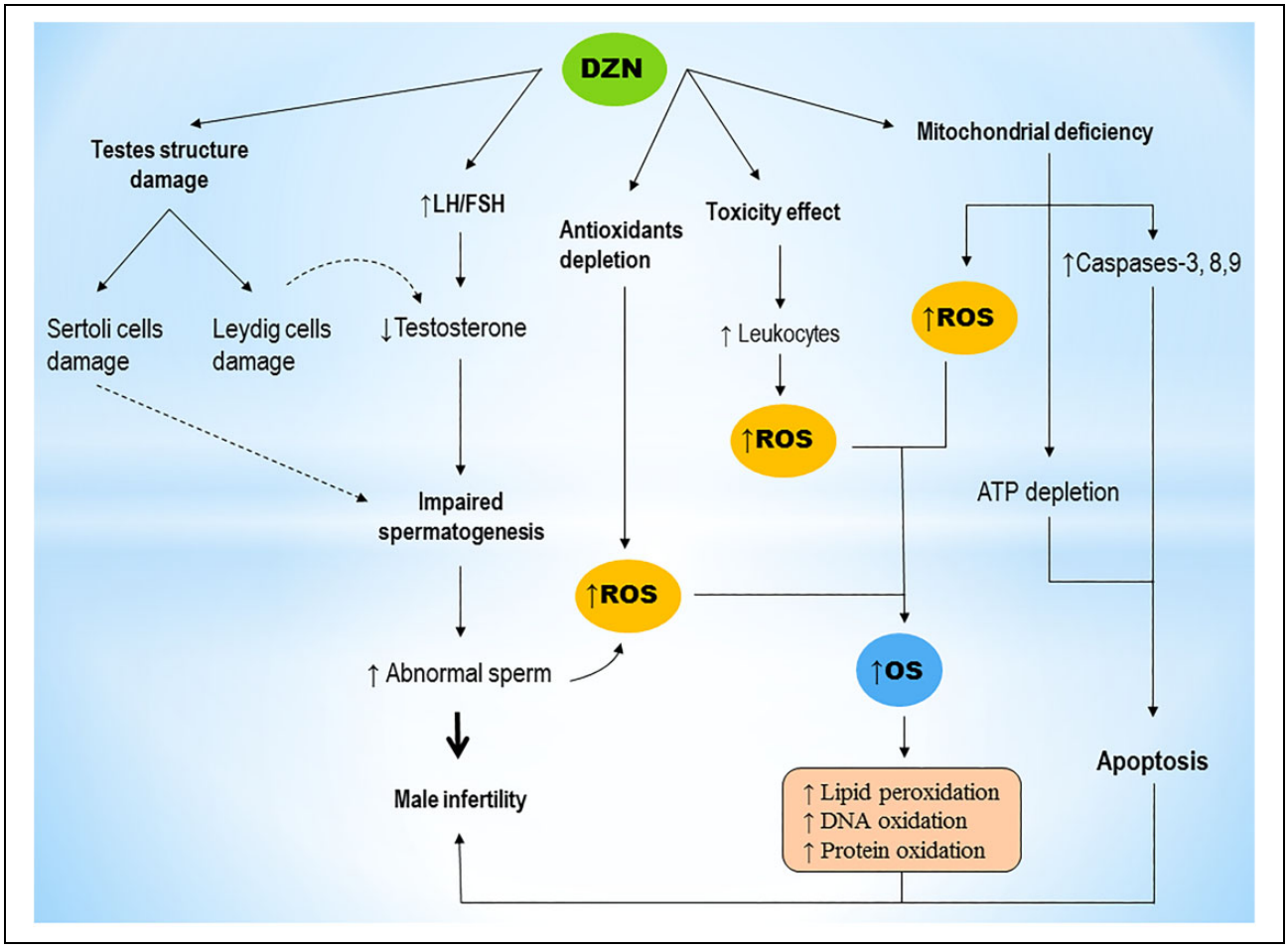


**Figure 1.** Mechanisms for the effects of DZN on impaired spermatogenesis and male infertility. DZN decreases spermatogenesis and leads to male infertility through several mechanisms, including massive ROS production and OS, reduced activity of antioxidants, testicular cell damage, decreased production and secretion of testosterone, mitochondria deficiency, inflammation, and apoptosis. DZN: diazinon; ROS: reactive oxygen species; OS: oxidative stress; LH: luteinizing hormone; FSH: follicle-stimulating hormone.

spermatogenesis. The effect of OS will be further discussed in the following sections.

## Semen quality

DZN is now considered one of the significant insecticides that increases poor semen quality and sperm aneuploidy in men (Swan et al., 2003). Many studies have proposed that maturing spermatozoa are possible targets of DZN effect in the testis. Pina-Guzmán et al. (2005) showed that spermatozoa are very sensitive to DZN during the late steps of maturation. They also revealed that DZN administration (8.12 mg/kg) causes severe damage to mice spermatozoa (Pina-Guzman et al., 2005). Decreased numbers of germ cells, spermatogenic cells, spermatocytes, and spermatids also have been reported in previous research (Fattahi et al., 2009; Hatjian et al., 2000; Maitra and Sarkar, 1996; Salem, 1998). As germ cells are a critical step in the spermatogenesis process, decreases in the number of progenitor cells can inhibit the production of viable spermatozoa. Toman et al. (2016) investigated the effect of DZN exposure on rat sperm motility by computer-assisted semen analysis. They observed that intraperitoneal exposure to DZN (20 mg/kg) is associated with reduced sperm motility, progressive motility, beat cross frequency (BCF), and increased amplitude of lateral head displacement (Toman et al., 2016). Similarly, Lifeng et al. (2006) observed a significant reduction in BCF following exposure to insecticide fenvalerate among occupational workers. Several studies have reported increased frequencies of sperm with abnormal morphology in DZN-exposed

individuals. In a previous study, Abd el-Aziz et al. (1994) found increased percentages of dead and morphologically abnormal spermatozoa in DZN-treated male rats. Toman et al. (2016) illustrated that DZN affects the sperm morphology with increases mainly in tail abnormalities. Increased frequency of sperm aneuploidy was also reported in men occupationally exposed to OP derivatives such as ethyl-parathion, methamidophos, and DZN (Akturk et al., 2006; Recio et al., 2001). Therefore, deterioration of sperm quality and decreases in sperm motility, counts and normal morphology can negatively affect fertilization success and induce male infertility (Figure 1).

## Sperm DNA damage

Sperm chromatin and DNA damage at different stages of spermatogenesis is now considered one of the other mechanisms by which DZN induces male infertility (Salazar-Arredondo et al., 2008). Although sperm DNA integrity is protected by its highly compacted structure, a great number of studies have revealed that DZN can induce severe DNA damages (Pina-Guzman et al., 2005; Salazar-Arredondo et al., 2008; Zhang et al., 2012). Epidemiological studies have shown sperm chromatin and DNA alterations in men exposed to several OPs (Akturk et al., 2006; Recio et al., 2001; Sanchez-Pena et al., 2004). Pina-Guzman et al. (2005) demonstrated an alteration in sperm chromatin condensation and DNA damage during late spermatid differentiation through the epididymis after acute exposure to single doses of DZN. Several *in vivo* studies reported that DZN alters sperm chromatin and DNA and promotes local apoptosis (Salazar-Arredondo et al., 2008; Sarabia et al., 2009). Epigenetic modifications may be also another mechanism of DZN effect on DNA damage. A study showed that DZN can modify gene promoter with DNA methylation (Zhang et al., 2012). DZN-induced DNA fragmentation in cells deriving from large intestine, liver, and kidney was also shown in previous studies (Boussabbeh et al., 2016; Tsitsimpikou et al., 2013). Since sperm chromatin condensation and DNA integrity are critical for the proper transmission of paternal genetic information, DZN toxicity can be associated with irreversible changes in sperm chromatin structure, defects in fertilizing ability, and embryo development (Figure 1). DZN-induced OS may be a main reason for sperm DNA fragmentation and chromatin abnormalities in exposed individuals.

## Endocrine disruption

Recent evidence has revealed that DZN can also disturb levels of sex hormones, which are critical for the regulation and initiation of spermatogenesis. Gonadotropins (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) and testosterone are the main regulators of germ cells development and spermatogenesis. The action of LH is mediated through the production of testosterone by the Leydig cells (Simoni et al., 1999). FSH and testosterone act through the Sertoli cells and stimulate all phases of spermatogenesis. FSH is also essential for the development of Sertoli cells and for induction and maintenance of normal spermatogenesis (Ramaswamy and Weinbauer, 2014). Therefore, abnormal spermatogenesis may be often associated with altered contents of serum gonadotropins and testosterone. Several lines of studies have shown significant changes in plasma levels of gonadotropins and testosterone in DZN-exposed subjects (Sarkar et al., 2000; Shan et al., 1995). Fattahi et al. (2009) demonstrated that DZN administration can have adverse effects on reproductive function by decreasing the mass of testis and testosterone level and increasing the concentrations of LH and FSH. Some studies indicated that DZN can enter the pituitary gland and lead to increases in circulating LH and FSH levels by suppressing the negative feedback at the anterior pituitary (Fattahi et al., 2009). Increased levels of serum LH are associated with germinal cells disruption and spermatogenesis deficiency (Ibrahim and El-Gamal, 2003; Izumi et al., 2005). Civen and Brown (1974) suggested that OPs can decrease serum steroid hormone levels by increasing steroid catabolism and inhibition of steroidogenesis. In another study, Chattopadhyay et al. (2005) illustrated that OPs can inhibit steroidogenesis in adrenal cells. Given the regulatory function of testosterone for differentiation of sex organs and spermatogenesis, maintenance of testosterone levels is critical for normal spermatogenesis and fertility (Pidoux et al., 2007; Watanabe et al., 1986). Therefore, decreases in serum testosterone levels induced by DZN may cause impaired spermatogenesis and infertility (Abd el-Aziz et al., 1994) (Figure 1).

## Mitochondrial deficiency

Mitochondria are critical for sperm normal function and fertilization process. They generate ATP and ROS, which are needed for proper sperm function and capacitation (Amaral et al., 2013). They also serve as

intracellular $Ca^{2+}$ stores, and their membrane potential is crucial for energy maintenance of sperm motility (Piomboni et al., 2012). Therefore, defects in sperm mitochondria can be correlated with massive production of ROS, OS, loss of sperm function, and fertilization rate.

Many experimental studies have revealed that DZN can induce mitochondrial damage through the loss of mitochondrial membrane potential and decreased mitochondrial activity (Aluigi et al., 2010). Shiri et al. (2016) demonstrated that DZN treatment caused a 46% decrease in the percentage of mitochondrial activity and a 23% decline in mitochondrial membrane potential, which was associated with increased activities of caspase-3 and caspase-9 and subsequently apoptosis. Mitochondrial damage triggers the caspase cascade leading to increased apoptosis. Razavi et al. (2013) indicated that DZN promotes release of cytochrome C from mitochondria to the cytosol, which in turn is associated with higher activity of caspase 3, Bax protein, and eventually apoptosis. In another study, Aluigi et al. (2010) considered DZN as a specific biomarker of DZN toxicity that increases the loss of mitochondrial potential and apoptosis in a dose-dependent manner. Therefore, these data suggest that mitochondrial damage plays a critical role in DZN-induced cell death, which can affect sperm motility, capacitation, and fertility rate (Figure 1).

## Apoptosis

Recent studies have indicated that apoptosis is a main cytotoxic mechanism of DZN on sperm cells (Bagherpour Shamloo et al., 2016). Several lines of experimental studies have demonstrated that cell incubation with DZN resulted in the reduction of cell viability, as well as apoptosis and necrosis (Boussabbeh et al., 2016; Khaksar et al., 2017; Lari et al., 2015; Shiri et al., 2016). DZN-induced cell death and apoptosis also have been reported in other cell types, including ovarian follicular cells, cardiac muscle cells, peripheral blood lymphocytes, and skeletal muscles (Aluigi et al., 2010; Pournourmohammadi et al., 2005). Increased activities of caspases-3, -8, and -9 as well as increased contents of Bax and reduced levels of Bcl-2 (enhanced Bax/Bcl-2 ratio) are considered as one of the main mechanisms by which DZN induces apoptosis (Boussabbeh et al., 2016). Caspase-3, which is released after cell damage, induces apoptosis, while Bcl-2 suppresses the apoptotic response (Truong-Tran et al., 2001). In a recent study, Boussabbeh et al. (2016) have revealed that DZN induces caspases activation and cell death.

OS induced by ROS is another significant mechanism by which DZN stimulates apoptosis (Colovic et al., 2015). Recent studies have reported that increased level of ROS and OS in seminal plasma of infertile patients is associated with higher levels of caspase-3 and apoptosis (Agarwal and Said, 2005; Wang et al., 2003). ROS are highly reactive free radicals that cause cell damage and apoptosis through the oxidation of cellular lipids, proteins, and DNA (Colagar et al., 2009b). They also lead to cell damage and apoptosis through the induction of caspases, as the primary drivers of apoptosis, and release of mitochondrial cytochrome c (Layali et al., 2015). Tumor necrosis factor-$\alpha$ (TNF-$\alpha$), which has been reported in DZN-exposed subjects, is another factor that induces apoptosis (Ahmadi-Naji et al., 2017). Therefore, DZN exposure can be associated with apoptosis of sperm cells in early stages of spermatogenesis, as well as the spermatocyte maturation stage, resulting in hypogonadism, poor sperm quality, and male infertility (Figure 1).

## OS and inflammation

Increased production of ROS and OS can be considered as the major molecular and cellular mechanism of DZN toxicity on poor sperm quality and male infertility (Fattahy et al., 2007). OS is defined as a disturbance in the balance between the production of ROS and cellular antioxidant defense systems (Colagar and Marzony, 2009). ROS, which has been reported in semen of 25–40% of infertile patients, are highly oxidizing molecules that can interact with DNA, proteins, and unsaturated fatty and cause severe abnormalities in spermatozoa (Agarwal et al., 2014a; Colagar et al., 2009a).

Human spermatozoa are very susceptible to ROS because of the high concentration of polyunsaturated fatty acids (PUFAs) in their plasma membrane (Makker et al., 2009). PUFAs are essential for the fluidity of sperm membrane, ion transport, and events that occur during the capacitation, oocyte fusion, acrosome reaction, and fertilization process in the female reproductive tract. ROS-induced peroxidative damages may also deplete cellular ATP resulting in decreased phosphorylation of axonemal proteins and transient impairment of motility, as well as decreased sperm viability (Agarwal et al., 2014b). Therefore,

ROS-induced membrane lipid peroxidation can decrease the fluidity of sperm, membrane transport, survival of spermatozoa, sperm counts, motility, normal morphology, and male fertilization potential (Tahmasbpour et al., 2014). ROS can also target sperm DNA by causing DNA fragmentation, base modification, DNA strand breaks, deletions, frameshift mutations, and chromatin cross-linking (Aitken et al., 2010; Bellver et al., 2010; Tahmasbpour et al., 2014; Wright et al., 2014; Zribi et al., 2011). DNA damage can be associated with germ cells apoptosis and impaired spermatogenesis, leading to decreases in sperm counts and male infertility (Singh et al., 2003).

Although sperm mitochondria serve as an ROS generator in order to promote capacitation and acrosome reaction, leucocytes and morphologically abnormal spermatozoa or immature sperm cells are the other sources of ROS generation in human semen (Agarwal and Sekhon, 2011; Colagar et al., 2007). Therefore, increased number of these cells in male reproductive organs can be associated with excessive production of ROS, increased OS, poor quality of spermatozoa, and infertility. Many studies have revealed that DZN administration is associated with increased values of leukocytes, proinflammatory responses, and oxidative damages (Ahmadi-Naji et al., 2017; Moallem et al., 2014; Tsitsimpikou et al., 2013). In a more recent study, Ogasawara et al. (2017) have suggested that DZN can activate macrophages and enhance proinflammatory responses (Ogasawara et al., 2017). They have shown that DZN not only enhances the number of macrophages and production of proinflammatory markers such as IL-6 and TNF$\alpha$, but also it increases the expression of cyclooxygenase (COX)-2 and inducible nitric oxide synthase enzymes as a major source of ROS (Ogasawara et al., 2017). Similarly, Hedayati and Hassan Nataj Niazie (2015) and Zeinali et al. (2017) observed that DZN treatment significantly increases the number of leukocytes, especially lymphocytes and neutrophils. These data suggest that DZN-induced toxicity can accumulate inflammatory cells including macrophages and neutrophils with a subsequent release of chemical mediators of inflammation such as interleukins and growth factors that can recruit and activate other leukocytes in reproductive system. Activated leukocytes can produce high levels of ROS, which in turn may overwhelm the antioxidant defense systems, resulting in OS.

Numerous studies have illustrated that DZN causes oxidative damages to DNA, proteins, and lipids (Boussabbeh et al., 2016; Pakzad et al., 2013; Shah and Iqbal, 2010). Ahmadi-Naji et al. (2017) observed that exposure to DZN is associated with increased contents of lipid peroxidation (malondialdehyde) and protein oxidation (protein carbonyl) biomarkers. In another study, Boussabbeh et al. (2016) indicated that the acute and chronic exposure to OPs is significantly correlated with the enhanced production of ROS and lipid peroxidation in cells deriving from large intestine. Oksay et al. (2013) showed that DZN induces OS in rat testis by increasing lipid peroxidation levels and reducing glutathione (GSH), vitamin C, and vitamin E contents. Therefore, overproduction of ROS by DZN-induced phagocyte cells causes oxidative damage to sperm DNA, protein, and membrane PUFA, which may be correlated with impaired spermatogenesis, apoptosis, and low quality of sperm (Figure 1).

DZN not only induces ROS generation through mitochondrial deficiency (Brimfield et al., 2012; Kumar et al., 2015), but it also increases the number of morphologically abnormal spermatozoa, which are the main sources of ROS in seminal plasma. Therefore, the increased number of abnormal sperm cells affects their mitochondrial function and subsequently elevates production of ROS, which in turn influences sperm function (Agarwal et al., 2014b; Henkel, 2011).

Another important mechanism by which DZN can increase OS is modulated by its negative effects on seminal plasma antioxidants or enzymes that reduce the other antioxidants (Figure 1). Recent data have illustrated that DZN can reduce the antioxidant capacity of cells and disturb cellular redox capacity. For instance, Shiri et al. (2016) have reported that DZN causes a significant reduction in thiol molecules such as GSH. GSH is a cofactor for several antioxidant enzymes such glutathione-S-transferase (GST) and glutathione peroxidase (GPX). Interestingly, decreased activity of GPX and GST was reported after DZN treatment (Beydilli et al., 2015). Some studies showed that treatment with GSH prodrug such as *N*-acethylcysteine can reduce OS and toxicity induced by DZN (Oksay et al., 2013). Several studies found that chronic exposure to DZN can reduce the activity of GST-$\alpha$3 enzyme (Pourtaji et al., 2016; Sastry and Malik, 1982). This enzyme is critical in the cellular detoxification of xenobiotics. It catalyzes the conjugation of toxins with GSH and produces less toxic and more hydrophilic products that can then be partially metabolized and excreted (Lasram et al., 2014). Fujioka and Casida (2007) indicated the crucial role of GST-3$\alpha$ in OPs detoxification. In a more recent

study, Ahmadi-Naji et al. (2017) have shown that DZN decreases the activity of superoxide dismutase and catalase enzymes. Reduced contents of some low molecular antioxidants such as vitamin C, vitamin E, and $\beta$-carotene were observed after DZN exposure (Oksay et al., 2013). Recent evidence has revealed that DZN may induce OS through the downregulation of peroxiredoxin-6 (PRDX6) (Lari et al., 2014; Pourtaji et al., 2016). PRDX6 is an important antioxidant enzyme that reduces cellular hydrogen peroxide ($H_2O_2$). It also produces arachidonic acid using Phospholipases A2 (PLA2), which is critical in apoptosis-mediated TNF-$\alpha$. Therefore, down-expression of PRDX6 by DZN can promote cellular susceptibility to $H_2O_2$-induced apoptosis (Kim et al., 2011). In other research, Pourtaji et al. (2016) demonstrated that DZN reduces the expression of 3-mercaptopyruvate sulfurtransferase (MPST). MPST is a cellular antioxidant enzyme that plays an important role in regulation of the redox system (Nagahara et al., 2013). Therefore, overproduction of ROS induced by DZN toxicity in reproductive system can decrease the effective concentration of different enzymatic and nonenzymatic antioxidants, increasing the harmful effects of ROS on spermatozoa that are associated with abnormal sperm parameters. Hence, seminal plasma of DZN-treated individuals may be extremely sensitive to decreases in body levels of antioxidants.

## Conclusion

DZN causes a wide variety of structural and functional defects in the male reproductive system, including testicular lesions, Sertoli and Leydig cell damage, disturbances in the levels of sex hormones, mitochondrial deficiency, impaired spermatogenesis, reduced sperm quality, and infertility. It causes reproductive dysfunction through multiple cellular and molecular mechanisms. Excessive production of free radicals and OS can be considered as the main mechanism by which DZN directly contributes to sperm DNA fragmentation, membrane lipid peroxidation, protein oxidation, and consequently apoptosis and cell death. It induces OS in the reproductive system with disruption of mitochondria, increased activity of ROS-producing enzymes, depletion of enzymatic and nonenzymatic antioxidants, accumulation of leukocytes at the site of reproductive tissue, and inflammation reactions, resulting in imbalances in production and detoxification of ROS. Therefore, treatments with antioxidants may be valuable to protect reproductive function against DZN-induced damage.

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