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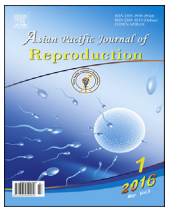

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Relationship of oxidative stress with male infertility in sulfur mustard-exposed injuries

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ABSTRACT

Sulfur mustard (SM) is a cytotoxic and chemical agent that targets different tissues such as reproductive system. SM causes a wide variety of pathological effects on reproductive system such as disturbance in reproductive hormones, testis atrophy, spermatogenesis deficiency, low quality of sperm and fertility problem. However, molecular and cellular mechanisms of its adverse effects are still not well known. General events such as tissue damage, inflammation, DNA alkylation, cell membrane defects, apoptosis and cell death are observed frequently in SM-exposed subjects. Oxidative stress (OS) and antioxidants depletion induced by SM seem to be one of the main factors that lead to low sperm quality and male infertility among exposed patients. It is believed that SM can trigger several molecular and cellular pathways linked to OS and inflammation in reproductive system that can cause impaired spermatogenesis, sperm apoptosis and poor sperm quality as well as loss of tissue structure and function. Identification of these signaling pathways and molecules gives us valuable information regarding the mechanisms of SM effect on reproductive dysfunction and the way for developing a better clinical treatment. Therefore, in this review we aimed to discuss the proposed cellular and molecular mechanisms of SM effect on reproductive system, the significance of oxidative stress and the mechanisms by which SM induces OS and antioxidants depletion in SM exposed men.

1. Introduction

2,2′-Dichlorodiethyl sulfide, commonly known as sulfur mustard (SM), is an oily lipophilic liquid which has been used as a chemical warfare agent. It is one of the major chemical warfare agents developed and used during World War I (1914–1919) [1]. But the highest unconventional application of SM occurred in Iran–Iraq war (1980–1988). During that period, it injured more than one hundred thousand Iranians, one-third of whom are still suffering from late effects [2,3]. This gas has several pathological consequences on various organs and systems of the victims which has previously been reported [4]. Eyes, skin and respiratory system are the main target organs of SM toxicity [5–7]. Other major acute pathological findings of SM exposure in humans include immunological and neuropsychiatric changes, gastrointestinal (GI) effects, hematological effects, sleep disorders and cancer [2,8–11]. Finally, it can induce a wide variety of genetic mutations, genetic damage and particularly lead to increased rates of cancer [12–15].

Reproductive system is one of the main targets of SM toxicity following exposure. Prevalence of infertility among SM exposed men has been reported from 2.5% to 35% [16–18]. Increased follicle stimulating hormone (FSH) levels along with decreased levels of testosterone and reduced semen quality were reported as the major effects after SM exposure [19–22]. An increased rate of fetal death and altered sex ratio were also reported in progenies of Iranian survivors of chemical attacks that included SM [16,19]. Although several studies have shown the negative effects of SM on reproductive function and male infertility, cellular and molecular mechanisms by which SM affects spermatozoa and induces poor sperm quality are still not well known. Therefore, there is a need for further detailed studies with focus on underlying mechanisms by which SM induces reproductive dysfunction and male infertility. One of these mechanisms is likely related to increased seminal plasma oxidative stress (OS) induced by reactive oxidative species (ROS). Recent studies have shown that pathological effects of SM are primarily due to its ability to form adducts with a variety of macromolecules such as DNA, lipids and proteins [23]. This can lead to inhibition of nucleic acid and protein biosynthesis, as well as ATP production which disruption of

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intracellular energy metabolism. It is well documented that SM accelerates oxidative stress through either an increase ROS generation from endogenous or a decrease in antioxidant capabilities and oxidative DNA repair [24]. This oxidative stress then, in turn, may damage DNA resulting in chromosome instability, modify gene expression, genetic mutation or modulation of cell growth that may result in cell death [25,26]. Therefore, toxicity from SM on cells may be the result of the direct damage induced by alkylating cellular components or SM-induced ROS production and oxidative stress.

In contrast with other cells, human spermatozoa are particularly susceptible to oxidative stress induced ROS. So, they are the major candidates for pathological and cytotoxic effects of SM [27]. In the following sections, we will discuss general reproductive effects of SM as well as significance of oxidative stress and mechanisms by which SM induces oxidative stress and antioxidants depletion in reproductive organs.

2. Reproductive effects

Although a small number of studies have considered the adverse effects of SM on reproductive function over the past few years, data addressing the negative effects of SM on sperm quality and male infertility are increasing. Several clinical investigations and animal experiments suggest that SM causes a wide variety of structural and functional defects in reproductive system including disturbances in the levels of reproductive hormones, testicular damages, sexual dysfunction, genital lesions, impaired spermatogenesis, poor sperm quality, and reduced fertility [19]. Some evidences addressing toxic effects of SM on reproductive function are summarized in Table 1.

Several studies have shown that SM exposure causes poor sperm quality, suggesting spermatozoa are particularly susceptible to toxic effects of SM. Azoospermia and severe oligospermia have been reported in 42.5% and 57.5% of patients with a history of exposure to SM, respectively [32]. Abnormal morphology of sperm (53.8%), decreased sperm motility (48.4%), reduced sperm count (23.1%) as well as abnormal semen viscosity (17.6%) and decreased semen volume (16.5%) have been reported as the most common semen abnormalities in patients exposed to SM [18]. In a study, semen analysis was considered among patients who had been exposed to SM during the Iran–Iraq war. The results of this analysis indicated the sperm abnormalities in 38% of the SM victims [18]. In another study, long-term toxic effects of SM on the testis and male fertility were investigated two decades after exposure. Male factor infertility was diagnosed in 23% of exposed patients and all semen indices were significantly decreased in the SM exposed men [21].

Several studies have revealed that SM can also disturb levels of reproductive hormones, which are essential for the regulation and initiation of spermatogenesis. Moreover, it has been found to interfere with the hypothalamus-hypophysis-testis axis, which is associated with impaired spermatogenesis and low quality of sperm. Gonadotropins (FSH, LH) and testosterone are the main regulators of germ cell development and spermatogenesis. Therefore, abnormal spermatogenesis is often associated with altered levels of serum gonadotropins and testosterone. Recent studies have revealed significant changes in plasma gonadotropins and testosterone concentrations among SM exposed

Table 1
Toxic effects of SM on male reproductive system.

Study model	Dose	Duration	Effects	References
SM victims	–	Several years	↓ Infertility (23.3%); ↓ Sperm quality (38.7%); ↑ Abortion (13.6%); ↑ Sexual dysfunction (9%); ↓ Libido (30%); ↑ Premature ejaculation (23.6%); ↑ FSH (57.6%); ↑ LH (66.3%)	[28,29]
SM victims	–	1st week after exposure	↓ Free serum testosterone; ↓ Dehydroepiandrosterone (DHES)	[30,31]
SM victims	–	5th week after exposure	↓ Free serum testosterone; ↓ Dehydroepiandrosterone (DHES)	[20]
SM victims	–	3rd and 5th week after exposure	↑ Serum FSH; ↑ Serum LH	[20]
SM victims	–	3 years after exposure	↓ Free serum Testosterone; ↑ Testicular atrophy; ↓ Spermatogenesis; ↑ Sertoli cell only pattern	[20,32,33]
SM victims	–	20 years after exposure	Normal LH, FSH and Testosterone	[21]
SM victims	–	3 months after exposure	↑ Oligozoospermia (33.3%)	[20]
SM victims	–	4 years after exposure	↑ Sperm counts (172×10^6)	[21]
SM victims	–	10 years after exposure	↑ Abnormal sperm (38%); ↑ Abnormal morphology of sperm (54%); ↓ Sperm motility (48%)	[18]
SM victims	–	15 years after exposure	↑ Oligozoospermia (10%)	[16]
SM victims	–	20 years after exposure	↓ Semen volume; ↓ Sperm counts; ↓ Sperm motility; ↓ Normal morphology of sperm	[21,28,34]
SM victims	–	20 years after exposure	↑ Sperm DNA damages	[35]
SM victims	–	8 years after exposure	↓ Libido (33.3%); ↑ Erectile dysfunction (9%); ↑ Premature ejaculation (23.6%)	[29]
SM victims	–	Few hours or few days after exposure	↑ Genital lesions; ↑ Hypopigmentation	[3,36]
Male rats	0.5 mg/kg	10 days	↑ Abnormal sperm; ↓ Sperm counts; ↓ Sperm motility	[37]
Male rats	5 mg/kg 10 mg/kg	10 days	↑ Abnormal sperm; ↓ Sperm counts; ↓ Sperm motility; ↓ Free serum testosterone; ↓ Testis weight	[38]

patients [30–32]. For example, increased level of FSH was found in plasma of patients with a history of SM exposure [20,21]. A long-term study by Azizi *et al*., demonstrated that exposure to SM results in very low androgen levels and hypo-responsiveness to GnRH. Serum total and free testosterone (FT) and dehydroepiandrosterone were markedly decreased after exposure to SM [20]. A significantly lower frequency of serum FT was found among SM induced patients (32.6%) [39]. In addition, sperm counts are positively correlated with the testosterone level. A marked reduction in intratesticular testosterone concentrations seems to be an important initiator of germ cell apoptosis in the seminiferous epithelium [33,40]. Therefore, any reduction of testosterone level by SM would be expected to interfere with the initiation of spermatogenesis, and lead to an increase of germ cell apoptosis and low quality of sperm. Furthermore, low sperm counts and the percentage of sperm abnormality are shown to be significantly associated with a high FSH level. An elevated FSH level is indicative of abnormal spermatogenesis and may indicate primary testicular failure. These findings suggest that reduced sperm count in SM exposed patients is attributable to primary testicular injury; a proof supporting the idea of SM gonadotoxicity [21]. Nevertheless, it seems that serum levels of the reproductive hormones are within the normal range in SM-exposed men several years after the injury, which is dose dependent.

Several studies on testicular biopsies in SM exposed patients revealed complete or relative arrest of spermatogenesis, atrophy of the germinal epithelium, intact Sertoli cells, and normal-appearing Leydig cells [20,21,32,34]. Therefore, spermatogenesis seems to be the main target of gonadal injury caused by SM. Arrest of spermatogenesis in testicular biopsies of SM-exposed subjects provides some other pathologic effects such as low semen volume because of ejaculatory duct obstruction, as well as poor sperm quality. Sexual dysfunction is also reported among SM victims. In a study of 800 Iranian men exposed to SM, 35% of them reported decreased libido [28]. Erectile dysfunction and premature ejaculation were also observed in 9% and 23.3% of patients, respectively [29]. These complications can be related to the decreased level of serum testosterone. Genital lesions such as hyperpigmentation, xerosis, and scars were also observed at the sites SM-induced injuries [41–43].

Effects of SM exposure on the reproductive hormones and sperm quality have been also studied in animal models. For example, increase in the percentage of abnormal sperm and defects in spermatogenesis were detected in male rats exposed to 0.50 mg kg^{-1} SM [37,38]. Alterations in testicular tissue integrity and decrease in the testis weight were observed in male rates after intraperitoneal injection of SM [44,45]. In another study, intravenous injection of SM in male mice resulted in damage to the testes with inhibition of spermatogenesis [19,44]. Increased distance between the seminiferous tubules, presence of necrotic forms of spermatocytes, and necrotic cells in the lumen were detected eight weeks after SM-treated rats [19].

Although experimental and human studies have shown the negative effects of SM, cellular and molecular mechanisms by which SM affects reproductive function and male infertility are poorly understood yet. Spermatogenesis seems to be the major target in reproductive system which can be influenced by exposure to SM. Following SM exposure, intense cellular and molecular alterations occur in reproductive tissue. After the exposure, innate immunity induces adaptive immune system with pro-inflammatory mediators. If the apoptosis and necrosis rate increase, cell contents will be released into the extra cellular matrix and immune cells will be activated. Thus, it is essential to identify the cellular and molecular mechanisms by which SM leads to reproductive damage and then find effective strategies to mitigate its toxicity.

3. Cellular and molecular mechanisms of SM toxicity

Due to great lipophilic property, SM can enter into the body easily and quickly through the eyes, skin and respiratory systems [41]. Afterward, it can distribute systemically *via* circulation and affect other tissues and organs such as reproductive system. When SM is absorbed, it undergoes intramolecular cyclization to form a sulphonium ion, which in turn alkylates DNA, lipids and proteins, leading to DNA strand breaks and eventually cell death [36,46]. Subsequently, tissue responses such as synthesis and release of inflammatory mediators and tissue damage began to emerge [47]. Although SM alkylates numerous physiological molecules in cells and tissues, SM-induced DNA damage is the primary initiator of the cellular responses that leads to the clinical injuries [9]. SM induces structural changes in cellular DNA since it contains one alkylation site which can immediately attack unsaturated nitrogen groups of DNA [48]. Toxic effects of SM have been attributed to DNA modification, uncoiling in part with the formation of N7-(2-hydroxyethylthioethyl) guanine (7-HETE-G), 3-hydroxyethylthioethyl adenine and the cross-link, di-(2-guanin-7-yl-ethyl) sulfide. Therefore, direct interaction of SM with DNA not only leads to DNA strands breaks, genotoxic stresses, proteins or genome modifications, but also it causes modifications in DNA replication and transcription, cell cycle arrest and apoptosis or cell death [23]. Furthermore, SM can directly interact with proteins and interfere with their natural function via miss folding, oxidation, cross-linking and enzyme disability. Lipids are also per oxidized when being exposed to SM, and then free radicals will be released as byproducts of lipid peroxidation. It is supposed that oxidative stress induced by free radicals is one of the first and direct effects of SM exposure, which is followed by arrest of cell signaling pathways, cell membrane collapse and cell death.

Another mechanism that may be involved in tissue damage is nicotinamide adenine dinucleotide (NAD) depletion. After SM-induced DNA damage, several DNA repair pathways including poly (ADP-ribose) polymerase (PARP) pathway, base excision repair, nucleotide excision repair, non-homologous and joining will be activated. Recently, studies have shown that DNA strand breaks induce PARP activation that lead to NAD^+ or ATP depletion and stimulation of the $NADP^+$ dependent hexosemonophosphate shunt, which in turn enhances synthesis and release of proteases [49]. Increased protease expression and activation is associated with cell death and tissue injuries [50]. Some studies have proposed that after SM-DNA interaction, PARP synthesizes poly (ADP-ribose) chain that is recruitment signal for other repair enzymes. It is proposed that PARP may be a switcher between apoptosis and necrosis and may have regulatory function over apoptosis. If damage is not repairable, apoptosis will be followed and PARP will be cleaved. But if cell losses its energy sources due to high demands for ATP during repairing process, it will be necrotic. Cellular ATP depletion blocks cleavage of PARP by caspase-3 and then PARP continues its activity. Recent studies have demonstrated that PARP

also produces poly (ADP-ribose) (PAR) alone that induces signals for apoptosis and cell death (Figure 1) [51].

In addition to PARP activation and direct effects of SM on DNA, lipids and proteins, experimental evidences reported the roles of NF-κB, p53, p38, Fas, calcium and calmodulin in the molecular mechanisms of SM-induced cell death, inflammation, and injury [11,52]. Several studies have considered calmodulin and increases in intracellular Ca^{2+} levels as one the most well-known signaling molecules induced by SM exposure [53]. Calmodulin and increased Ca^{2+} are proposed to play a critical role in apoptosis and cell death (Figure 1). Cellular Ca^{2+} can be increased by protein kinase signaling pathways that leads to activation of phospholipase C (PLC) and generation of inositol triphosphate (IP3), which acts on Ca^{2+} channels to release it from intracellular stores [54]. The other mechanism involves oxidative stress in which reactive oxygen species (ROS) generated by SM exposure, react with Ca^{2+} transport channels in the endoplasmic reticulum, mitochondria, and cell membrane. These reactions damage the Ca^{2+} transport channels, which results in an influx of Ca^{2+} into the cytosol [55]. High levels of cytosolic Ca^{2+} not only induce proteases activity (such as Caspase 3, 7 and 9), but also it induces Phospholipases and Endonucleases activity which in turn degrade cellular proteins, lipids and DNA [56] (Figure 1).

Earlier studies showed that SM induces upregulation of FasL and Fas, as an apoptotic signaling, in injured cells [57]. It is stated that FasL and Fas induce the process of Caspase activation (Caspase 3, 7–9), which in turn leads to protein degradations and apoptosis. The other signaling molecules such as NF-κB, p38, and p53 are mediator factors that mediate numerous cellular responses such as inflammation, apoptosis, proliferation, differentiation, and tumorigenesis [58,59]. Several studies implicated that SM induces these mediators and leads to inflammation, apoptosis or cell death among exposed cells. The other potential mechanism of SM-induced cell death is related to rapid inactivation of sulfhydryl-containing proteins and peptides, such as glutathione. These sulfhydryl compounds are critical in maintaining appropriate oxidation–reduction state of cellular components. Glutathione is also thought to be critical in reducing ROS in the cell and preventing peroxidation and loss of membrane integrity [60] (Figure 1).

4. Role of oxidative stress in male infertility

Oxidative stress can be defined as the imbalance between bioavailability of ROS and cellular antioxidant systems that can lead to critical failure of biological functions and ultimately cell death [61]. ROS, especially superoxide anion ($O_2^{\bullet}$), and hydroxyl radicals ($OH^{\bullet}$) are highly reactive molecules that belong to the class of free radicals and produced by living organisms as a result of normal cellular metabolism [61,62]. They are unstable molecules with short half-life that can adversely affect certain cellular processes and modifies cell components, such as lipids, proteins, and DNA in high concentrations [63]. Nevertheless, small amounts of ROS are required for normal sperm function such as acrosome induction and sperm capacitation. Therefore, they exhibit a double edged sword role in cells.

Oxidative stress induced by ROS has recently been proposed as one of the major causes for poor sperm quality, sperm dysfunction and male infertility [64–66]. Indeed, spermatozoa were the first cell type reported to show potential susceptibility to OS [67]. Excessive ROS can be produced by immature spermatozoa and leukocyte cells originate from the prostate and seminal vesicles [68]. They can attack sperm membrane

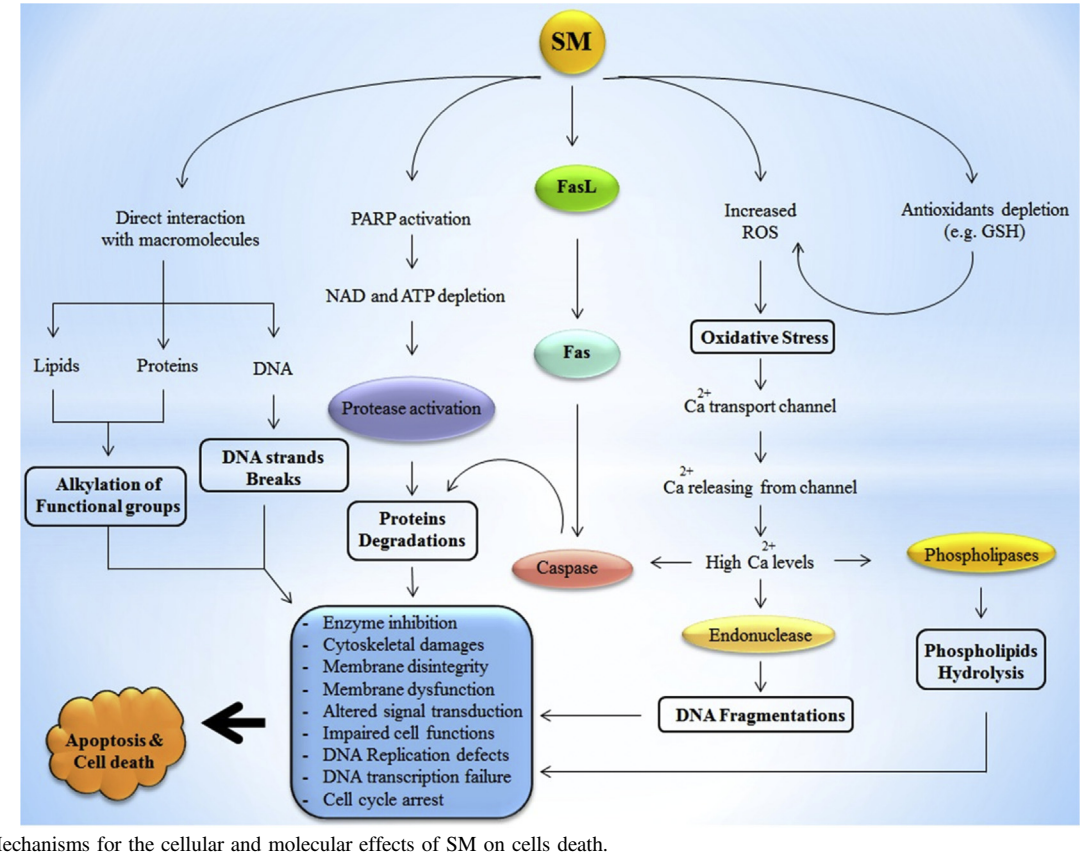

Figure 1. Mechanisms for the cellular and molecular effects of SM on cells death.

lipids, DNA and proteins; alter enzymatic systems; produce irreparable alterations; cause cell death; and ultimately, lead to decline in the semen parameters associated with male infertility [67].

ROS attacks the fluidity of sperm plasma membrane, with subsequent loss of the ability for oocyte fusion and fertilization [68]. Due to the high concentration of polyunsaturated fatty acids (PUFA) in the membrane of human spermatozoa, they are particularly susceptible to OS [69]. PUFA are responsible for the fluidity of sperm membrane, ion transport and the changes that occur during capacitation in female reproductive tract. Therefore, oxidation of PUFA by ROS causes to deficiency in membrane function and sperm death. Furthermore, decrease in fluidity could affect membrane transport activity and affect surviving of spermatozoa. A number of studies have shown that lipid peroxidation affects sperm concentration, motility, and normal morphology. Some studies have suggested that ROS attacks the integrity of DNA in sperm nucleus by causing base modification, DNA strand breaks, DNA fragmentation, deletions, frame-shift mutations, and chromatin cross-linking [70–74]. DNA damage included by excessive levels of ROS could accelerate process of germ cell apoptosis, leading to decline in sperm counts associated with male infertility [75]. Studies have found that the levels of ROS correlate with the motility of spermatozoa. Peroxidative damage to the sperm membrane and axonemal proteins seems to be the cause of permanent impairment in sperm motility because excessive ROS depletes ATP rapidly resulting in decreased phosphorylation of axonemal proteins and cause transient impairment of motility as well as decreased sperm viability [67]. Lipid peroxidation has also a deleterious effect on the ultramorphological status of the sperm cells and thereby on the male fertilization potential [74].

In order to counteract the toxic effects of ROS, human spermatozoa are equipped with antioxidant defense mechanisms and are likely to quench ROS, thereby protecting gonadal cells and mature spermatozoa from oxidative damage [67]. Furthermore, human seminal plasma contains enzymatic and low molecular weight antioxidants which make it able to as a free radical scavenger and hence it protects spermatozoa against ROS. This defense mechanism compensates the loss of sperm cytoplasmic enzymes when the cytoplasm is extruded during maturation [76]. Nevertheless, increased ROS in reproductive system can decrease the effective concentration of essential antioxidants, increasing the harmful effects of ROS on spermatozoa that are associated with abnormal sperm parameters [77]. Hence, seminal plasma is extremely sensitive to decrease in body levels of antioxidants.

5. SM induces oxidative stress

It is now proposed that oxidative stress induced by free radicals is one the major mechanisms for direct effects of SM exposure in human body. It appeared that oxidative stress induced with ROS is one reason for low sperm quality and male infertility among SM exposed patients. SM may increase ROS production in the testes, negatively impacting the sperm structure and function. A lot of studies have demonstrated that SM induces the process of oxidative damages in mustarded subjects [78–80]. SM leads to increased rate of oxidative stress in reproductive system with several mechanisms. One of these major mechanisms is related to high levels ROS that has destructive effect on normal cells and their functions. Over the past few years, extensive research has been carried out to establish a link between presence of leukocytes in the ejaculate and a male factor as the cause of infertility. Various studies point to a correlation between decreased sperm function and seminal plasma with abnormally elevated levels of ROS, IL-6, IL-8, and tumor necrosis factor, all of which result in increased sperm cell membrane LPO [81–83]. Recent studies have reported that exposure to SM is associated with inflammatory reactions and oxidative injury at the site of damaged tissues [80,84,85]. Experimental studies revealed that SM induces secretion of proinflammatory cytokines, chemokines and growth factors, including TNFα, IL-α, IL-β, IL-6, IL-8, IL-13, IL-15, INF-γ, macrophage chemotactic protein (MCP)-1, matrix metalloproteinases (MMPs) in damaged tissues [86–88]. SM can accumulate inflammatory cells including macrophages and neutrophils with a subsequent release of chemical mediators of inflammation such as interleukins and cytokines that can recruit and activate other leukocytes in reproductive system. Activated leukocytes can generate high levels of ROS in a respiratory burst, which may overwhelm the antioxidant strategies, resulting in oxidative stress in seminal plasma. ROS produced by SM-induced phagocyte cells cause oxidative damage to sperm DNA, protein and membrane PUFA, which may be closely related to inflammations, impaired spermatogenesis, apoptosis and low quality of sperm [35]. Several studies have shown that SM induces mitochondrial dysfunction, a process associated with increased ROS production, DNA oxidation and decrease in intracellular antioxidants [77,89]. Sperm cells are rich in mitochondria because a constant supply of ATP is required for their motility. Therefore, presence of abnormal and immature spermatozoa in the semen significantly elevates production of ROS, which in turn affects its mitochondrial function and subsequently, sperm function such as motility [67,90]. SM has been found to impair spermatogenesis and induces sperm DNA damage. In a recent study, association between SM exposure and sperm DNA fragmentation has been investigated two decades after SM injury. A significant increase in sperm DNA fragmentation index was observed in SM patients, suggesting the risk of congenital abnormalities and genetic defects in SM-exposed veterans' offspring created by intracytoplasmic sperm injection (ICSI) technique [34,35].

Another important mechanism by which SM can increase OS is modulated by its negative effects on seminal plasma antioxidants or enzymes that reduce the other antioxidants. Reduced glutathione (GSH) is thought to be a primary target for SM because its level has been markedly reduced after SM exposure [91]. Further evidences revealed that SM–GSH metabolites deplete cellular GSH and increase intracellular ROS as well as OS markers including DNA, lipid and protein oxidations [91]. Several investigators have shown that GSH treatment or N-acetylcysteine (NAC), as a GSH prodrug, can reduce the OX and toxicity induced by SM [60,92,93]. NADPH cytochrome p450 reductase is another target for SM. It is a flavin-containing electron donor for cytochrome p450, as a major enzyme that has a critical role in mediating detoxification of SM and its metabolites [94]. Several investigators have demonstrated that SM not only has an inhibitory effect on reduction of cytochrome-c, but it also inhibits NADPH cytochrome p450 reductase activity and stimulates ROS formation [94]. SM can also target other

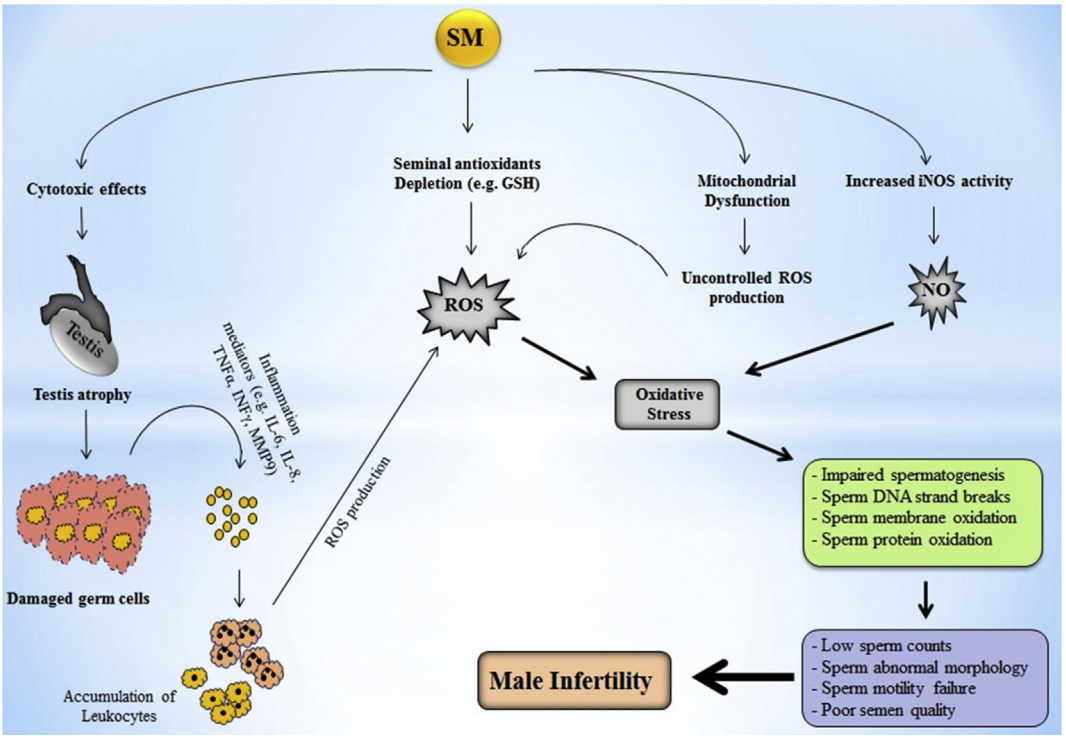

Figure 2. The effects of oxidative stress induced by SM on sperm cells and male infertility.

antioxidant enzymes such as thioredoxin reductase, catalases (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferases, which are critical for controlling cellular antioxidants balance [88,95]. Decrease in activity and effective concentration of these enzymes can occur as a result of SM-induced alkylation or changes in expression of these enzymes.

Besides ROS, reactive nitrogen species (RNS) such as nitrogen oxide (NO) can contribute to oxidative damage and toxicity when produced in excessive amounts. It is synthesized from arginine and oxygen mediated by nitric oxide synthase; however, its ability to damage cells is depended on local concentrations of NOS, metabolism into reactive intermediates, as well as its detoxification in target tissues [96]. NO is a strong oxidant which inhibits mast cell degranulation and histamine release. Massive production of NO probably triggers inflammation and apoptosis *via* increased regulation of iNOS activity [96]. Despite NO being a strong oxidant, but ROS derived $O_2^{\bullet}$ reacts with NO and forms a stable oxidant known as peroxynitrite ($ONOO^-$) [97]. This new molecule is not a radical, but it is a strong and stable oxidant that can interact with biomolecules and induces more damages [96,98] (Figure 2). In several studies, SM has been reported to modulate expression and activity of NOS and nitric oxide production in different tissues [98,99].

In conclusion, SM causes a wide variety of structural and functional defects in reproductive system including disturbances in the levels of sex hormones, testicular damage, sexual dysfunction, genital lesions, impaired spermatogenesis, poor sperm quality, and reduced fertility. It provides reproductive dysfunction with several cellular and molecular mechanisms; however, the majority of proposed roles for molecular and cellular events in SM injury remain mostly theoretical. SM exerts its toxicity through a number of pathogenic mechanisms including DNA alkylation, NAD depletion, antioxidants depletion, inflammation and cellular apoptosis. Oxidative stress induced by SM is one of the main mechanisms by which SM directly contributes to DNA fragmentation, lipid and protein oxidation and as the result sperm apoptosis. It induces OS in reproductive system with disruption of mitochondria, increases activity of enzymes producing ROS and seminal plasma antioxidants depletion including GSH and several antioxidant enzymes, accumulation of leukocytes at the site of reproductive tissue, inflammation reactions and as a result imbalance in production and detoxification of ROS. Therefore, treatments with antioxidants can be valuable to protect reproductive function against SM-induced damage. However, successful therapy for SM toxicity may depend on disease severity, antioxidants dosage, development of new antioxidants effective against SM-induced ROS and their improved delivery to target tissues.

Conflict of interest statement

We declare that we have no conflict of interest.

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