Toxicology Research

REVIEW

Cite this: *Toxicol. Res.*, 2018, **7**, 1029

Cellular and molecular mechanisms of sulfur mustard toxicity on spermatozoa and male fertility

Asghar Beigi Harchegani,[a] Mahdiyeh Mirnam Niha,[b] Milad Sohrabiyan,[a] Mahdi Ghatrehsamani,[c] Eisa Tahmasbpour *[d] and Alireza Shahriary *[a]

Received 23rd February 2018,
Accepted 9th July 2018

DOI: 10.1039/c8tx00062j

rsc.li/toxicology-research

Sulfur mustard (SM) is a toxic compound that can target human spermatozoa. SM induces a wide variety of pathological effects in human reproductive organs, including sexual hormone disturbance, testicular atrophy, impaired spermatogenesis, poor sperm quality, defects in embryo development, childhood physical abnormalities, and severe fertility problems. However, the molecular and cellular mechanisms of SM action on male reproductive health and human sperm function are unclear. Excessive production of reactive oxygen species and the resulting oxidative stress is likely a significant mechanism of SM action, and could be associated with sperm DNA damage, membrane lipid peroxidation, reduced membrane fluidity, mitochondrial deficiency, apoptosis, and poor sperm quality. In this review, we aim to discuss the cellular and molecular mechanisms of SM action on sperm and reproductive health, the significance of OS, and the mechanisms through which SM enhances the infertility rate among SM-exposed individuals.

Introduction

Sulfur mustard (SM) is a lipophilic compound that has been used as a chemical warfare agent. During the Iran–Iraq war of 1980–1988, the unconventional use of SM injured more than 100 000 Iranians, of which one-third still suffer from chronic effects.[1,2] Numerous studies have reported different pathological and clinical effects of SM exposure on various organs.[3] Although the eyes, skin, and airway are the primary targets of SM toxicity,[4–6] immunological, hematological, and neuropsychiatric abnormalities, gastrointestinal problems, and sleep disorders are the other main pathological findings.[1,7–10]

Reproductive organs are another significant target for SM toxicity. However, reports are still conflicting regarding the effect of SM on human sperm and male infertility. Previous studies have shown that the infertility rate in SM-exposed men ranges from 2.5% to 35%.[11–13] Sexual hormone disturbance, structural damage such as testicular atrophy, impaired spermatogenesis, and poor sperm quality are the effects on human reproductive health and fertility proposed to be caused by SM.[14] However, the actual mechanism through which SM triggers these abnormalities is poorly understood.

Excessive production of reactive oxidative species (ROS) and oxidative stress (OS) seems to be a significant mechanism of SM action on human reproductive function.[14] Recent studies have indicated that SM accelerates OS through the mass generation of ROS from endogenous sources or by decreasing antioxidant capabilities and oxidative DNA repair.[15] The resultant OS can also damage DNA, leading to chromosome instability, altered gene expression, apoptosis, and cell death.[16,17] SM can also form adducts with DNA, lipids, and proteins,[18] and suppress nucleic acid and protein biosynthesis, which is associated with ATP depletion and the disruption of intracellular energy metabolisms. Therefore, SM toxicity can result from the direct damage induced by the alkylation of cellular components or ROS overproduction and oxidative stress.

As human sperm membranes contain a higher percentage of unsaturated fatty acids relative to other cells, they are particularly susceptible to OS and ROS. Therefore, spermatozoa can be considered major candidates for the pathologic and cytotoxic effects of SM.[19] In the following sections, we will discuss the general reproductive effects of SM, the significance of OS, and the mechanisms through which SM induces ROS generation and antioxidant depletion in reproductive organs.

Gonadotoxicity effect of SM

Although only several studies have considered the negative effects of SM on human reproductive health, data on the

[a]*Chemical Injuries Research Center, Systems biology and poisonings institute, Baqiyatallah University of Medical Sciences, Tehran, Iran. E-mail: Shahriary961@gmail.com; Tel: +21-82482502*
[b]*Department of Medical Radiation Engineering, Central Tehran Branch, Islamic Azad University, Tehran, Iran*
[c]*Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*
[d]*Laboratory of Regenerative Medicine & Biomedical Innovations, Pasteur Institute of Iran, Tehran, Iran. E-mail: tahmasb62@gmail.com; Tel: +21-9111193051*

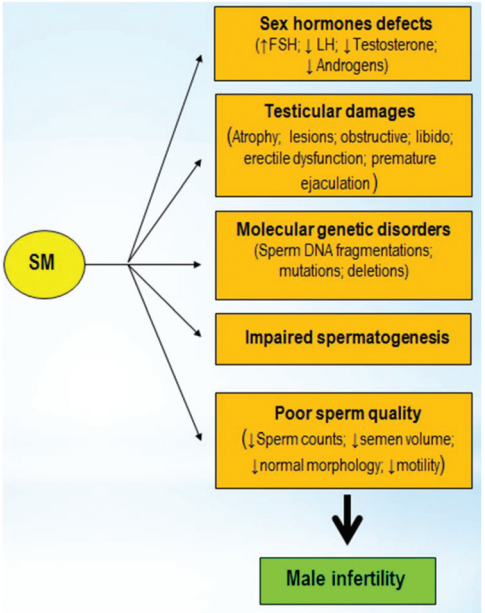

Fig. 1 SM affects the male reproductive system through several mechanisms, including sexual hormone disturbance, testicular damage, sperm DNA damage, impaired spermatogenesis, and poor sperm quality.

adverse effects of SM on sperm function and male infertility are increasing. A growing number of clinical investigations and experimental studies have shown that SM affects the male reproductive system through several mechanisms, including sexual hormone disturbance, testicular atrophy, sexual dysfunction, genital lesions, impaired spermatogenesis, and poor sperm quality[14] (Fig. 1). Table 1 shows a list of human and animal-based studies that considered chronic and severe effects of SM on male reproductive function and sperm quality.

Table 1 Gonadotoxicity effects of SM on male reproductive function

Study model	Times after exposure	Findings	Ref.
Human	Several years	↓Fertility rate (23.3%); ↓quality of sperm (38.7%); ↑abortion (13.6%); ↑sexual dysfunction (9%); ↓libido (30%); ↑premature ejaculation (23.6%); ↑sex hormone deficiency, ↑FSH (57.6%); ↑LH (66.3%)	24 and 25
Human	1st week	↓Free serum testosterone (FT); ↓dehydroepiandrosterone (DHES)	89
Human	5th week	↓FT; ↓DHES	20
Human	3rd and 5th week	↑Serum FSH; ↑serum LH	20
Human	3 years	↓FT; ↑testicular atrophy; ↑impaired spermatogenesis; ↑Sertoli cells only pattern	20 and 23
Human	20 years	Normal LH, FSH and Testosterone	21
Human	3 months	↑Oligozoospermia (33.3%)	20
Human	4 years	↑Total sperm counts	21
Human	10 years	↑Abnormal sperm (38%); ↑sperm with abnormal morphology (54%); ↓sperm motility (48%)	13
Human	15 years	↑Oligozoospermia (10%)	11
Human	20 years	↓Semen volume; ↓sperm counts; ↓sperm motility; ↓ sperm with normal morphology	21–23
Human	20 years	↑Sperm with DNA damages	81
Human	8 years	↓Libido (33.3%) ↑erectile dysfunction (9%); ↑premature ejaculation (23.6%)	25
Human	Few hours or few days	↑Genital lesions; ↑hypopigmentation	2 and 39
Male rats	10 days	↑Abnormal sperm; ↓sperm counts; ↓sperm motility	29
Male rats	10 days	↑Abnormal sperm; ↓sperm counts; ↓sperm motility; ↓FT; ↓testicular weight	90

Structural changes and impaired spermatogenesis

Several studies have shown that SM has a significant effect on the structure and function of the testes. Testicular biopsy of SM-exposed patients showed a complete or relative arrest of spermatogenesis and atrophy of the germinal epithelium, but normal Sertoli and Leydig cells.[20–23] These data suggest that spermatogenesis is a significant target of SM toxicity. Spermatogenesis deficiency in SM-exposed individuals can produce further pathologic effects, such as a low semen volume owing to ejaculatory duct obstruction and poor sperm quality. Sexual dysfunction is reported among SM victims. Pour-Jafari *et al.*[24] showed that, among 800 SM-exposed Iranian men, 35% had decreased libido.[24] A previous study reported erectile dysfunction (9%) and premature ejaculation (23.3%) in SM-exposed patients.[25] These complications might be due to decreased level of serum testosterone. Other studies reported genital lesions, such as hyperpigmentation, xerosis, and scars at the sites of SM-induced injuries.[26–28]

The effects of SM exposure on testicle structure and spermatogenesis have also been studied in animal models. For example, an increased percentage of abnormal spermatozoa and impaired spermatogenesis were observed in male rats exposed to 0.50 mg kg^{-1} SM.[29] Changes in testicular integrity and a decrease in testicular weight were detected in male rats after intraperitoneal injection of SM.[30,31] Other studies have reported that the intravenous injection of SM into male mice caused testicle damage and spermatogenesis deficiency.[30,32] Furthermore, an increased distance between seminiferous tubules, necrotic forms of spermatocytes, and necrotic cells in the lumen were found after eight weeks in SM-exposed rats.[32] Therefore, degenerative changes in testicular structure can be considered a main mechanism of SM action that might be associated with impaired spermatogenesis, decreased spermatozoa numbers, poor sperm quality, and eventual male infertility.

Sperm quality

Several studies have indicated that SM exposure results in poor sperm quality, which suggests spermatozoa are particularly susceptible to the cytotoxic effects of SM. For example, a previous study found azoospermia and severe oligospermia in 42.5% and 57.5% of SM-exposed patients, respectively.[23] Shakeri *et al.*[33] observed abnormal sperm morphology (53.8%),

reduced sperm motility (48.4%), low sperm count (23.1%), abnormal semen viscosity (17.6%), and declined semen volume (16.5%) in SM-exposed patients. In another study, semen analysis was performed on patients exposed to SM during the Iran–Iraq war. The results showed sperm abnormalities in 38% of SM victims.[13] In other research, the long-term effects of SM on the testes and male fertility were considered two decades after exposure. Male factor infertility was detected in 23% of SM-exposed patients and all semen indices were significantly decreased.[21] Therefore, these data suggest that spermatozoa are a possible target of SM effects in the testes.

Sexual hormone deficiency

SM exposure can disturb reproductive hormones that are critical for the regulation and initiation of spermatogenesis.[34] Furthermore, SM can interfere with the hypothalamus–hypophysis–testis axis, which is associated with impaired spermatogenesis and poor sperm quality (Fig. 2).

Gonadotropins, including follicle-stimulating hormone (FSH), and luteinizing hormone (LH), and testosterone, are key regulators of germ cell development and spermatogenesis. Altered expression and secretion of gonadotropins and testosterone can be associated with abnormal spermatogenesis and male infertility. Previous studies have shown significant changes in plasma levels of gonadotropins and testosterone in SM-exposed patients.[20,21,23,32,35] For example, increased levels of FSH were observed in the serum of patients exposed to SM.[20,21] In a long-term study, Azizi *et al.*[20] found that exposure to SM reduced androgen levels and hypo-responsiveness to GnRH. They also found that the total serum and free testosterone and dehydroepiandrosterone were markedly decreased after SM exposure.[20] In another study, Agin[36] found reduced serum free testosterone levels in 32.6% of SM-exposed patients.

As sperm counts are positively correlated with testosterone level, a marked reduction in intratesticular testosterone contents can initiate germ cell apoptosis in the seminiferous epithelium.[37] Therefore, any reduction in testosterone concentration caused by SM might interfere with the initiation of spermatogenesis, leading to germ cell apoptosis and low sperm quality. Furthermore, there is a significant relationship between a high serum FSH level with reduced sperm count and abnormal spermatozoal morphology.[20] Increased FSH levels are indicative of abnormal spermatogenesis and may suggest primary testicular failure. These findings indicate that a reduced sperm count in SM-exposed patients can be attributed to a primary testicular injury, which supports the idea of SM gonadotoxicity.[21] However, serum levels of reproductive hormones seem to be within the normal range in SM-exposed men several years after the injury, which is dose-dependent.[14]

Mechanisms of SM action

As SM is a lipophilic compound, it can be easily absorbed and quickly enter the body through the eyes, skin, and respiratory system.[26] SM then distributes systemically through the circulatory system and affects various organs, especially the reproductive system. Recent evidence has suggested that SM toxicity is mediated through several mechanisms, such as macromolecule damage, cellular nicotinamide adenine dinucleotide (NAD) depletion, increased cellular calcium levels, increased apoptosis mediators, oxidative stress, inflammation, and cellular antioxidant depletion[38] (Fig. 3).

Macromolecule damage

When SM is absorbed, it undergoes an intramolecular cyclization to form a sulfonium ion that can alkylate DNA, lipids, and

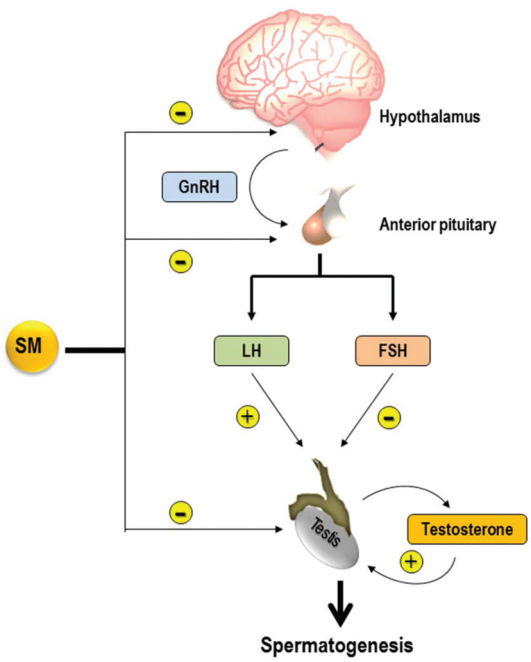

Fig. 2 Pathological effects of SM on the hypothalamus–hypophysis–testis axis, which disrupts reproductive hormones and spermatogenesis.

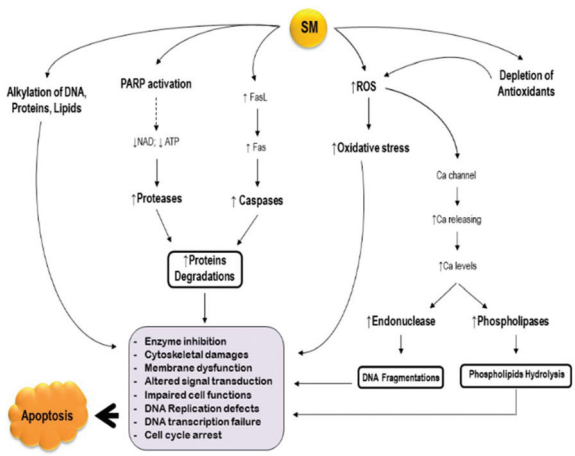

Fig. 3 Possible cellular and molecular mechanisms of SM action in apoptosis and cell death.

proteins, leading to DNA strand breaks and subsequent cell death.[39,40] These cellular effects are associated with tissue responses, such as the synthesis and secretion of inflammatory mediators, and tissue damage (Fig. 3).[41]

DNA damage is the primary initiator of the cellular responses associated with clinical injuries.[8] SM induces different structural modifications in DNA, which can lead to DNA strand breaks, genotoxic stress, protein or genome modification, deficient DNA replication and transcription, cell cycle arrest, apoptosis, and cell death.[18] SM can also directly interact with proteins and interfere with their normal function through misfolding, oxidation, cross-linking, and enzyme disability.[38] Lipids are also targets for SM that can undergo peroxidation when exposed to SM, with free radicals released as byproducts.[14]

Nicotinamide adenine dinucleotide (NAD) depletion

NAD depletion is another mechanism of SM action. Upon SM-induced DNA damage, DNA repair systems are activated, including the poly(ADP-ribose) polymerase (PARP) pathway, base excision repair, and nucleotide excision repair.[38] Recent studies have shown that DNA breaks induce PARP activation that leads to NAD^+ or ATP depletion and the stimulation of $NADP^+$-dependent hexose monophosphate shunt. This, in turn, enhances protease synthesis and release.[42] Increased expression and activation of proteases is associated with cell death and tissue injuries[43] (Fig. 3). Previous studies have also shown that the PARP produces poly(ADP-ribose) (PAR) alone, which induces signals for apoptosis and cell death.[44]

Calcium (Ca^{2+}) release

Recent studies have used calmodulin and increased intracellular Ca^{2+} levels as a signalling molecule induced by SM exposure.[45] Calmodulin and increased intracellular Ca^{2+} content play an important role in apoptosis and cell death (Fig. 3). Cytosolic calcium can be increased by the activity of protein kinase (PK) signalling pathways, leading to the activation of phospholipase C (PLC) and the generation of inositol triphosphate (IP3), which acts on calcium channels to release calcium from intracellular stores.[46] Another possible mechanism of cytosolic Ca^{2+} enhancement results from the massive production of ROS caused by SM. ROS react with Ca^{2+} transport channels inside the endoplasmic reticulum, mitochondria, and cell membrane. These interactions damage the Ca^{2+} transport channels, resulting in an influx of Ca^{2+} into the cytosol.[47] Increased contents of cytosolic Ca^{2+} not only induce protease (such as caspases) activity, but also phospholipase and endonuclease activity, which degrades cellular proteins, lipids, and DNA[48] (Fig. 3).

Apoptosis mediators

Previous studies have shown that SM induces the overexpression of FasL and Fas as an apoptotic signalling pathway in damaged cells.[49] FasL and Fas induce caspase activation, which leads to protein degradation and apoptosis (Fig. 3). The other signalling molecules, such as NF-κB, p38, and p53, are mediator factors that trigger numerous cellular responses, such as inflammation, apoptosis, proliferation, and differentiation.[50,51] SM induces these mediators and leads to inflammation, apoptosis, or cell death in SM-damaged cells.

Oxidative stress and male infertility

The mass generation of ROS and OS is likely a major reason for poor sperm quality and male infertility in SM-exposed patients. Oxidative stress is defined as the imbalance between ROS generation and cellular antioxidant systems.[52] ROS are highly reactive free radicals produced by living organisms during normal cellular metabolism.[52,53] At high concentrations, they can interact with lipids, proteins, and DNA and adversely affect certain cellular processes and modify normal cell functions.[54] However, ROS are critical for normal sperm function, such as the acrosome reaction and sperm capacitation at low concentrations.[55]

OS has been proposed as a main reason for low sperm quality and male infertility.[55–57] Recent studies have shown that immature spermatozoa or abnormal sperm cells and leukocytes are the major sources of ROS in human semen.[58] ROS target sperm membrane lipids, DNA, and proteins, alter enzymatic systems, produce irreversible alterations, cause cell death, and, ultimately, lead to a decline in the semen parameters associated with male infertility[58] (Fig. 4).

ROS can decrease the fluidity of the sperm plasma membrane, leading to loss of the sperm ability for oocyte fusion and fertilization.[59] As human spermatozoa contain a high percentage of polyunsaturated fatty acids (PUFA) in their plasma membrane, they are highly susceptible to ROS.[60] PUFA are critical for sperm membrane fluidity, ion transport, and sperm capacitation within the female reproductive tract. Therefore, sperm lipid peroxidation negatively affects membrane func-

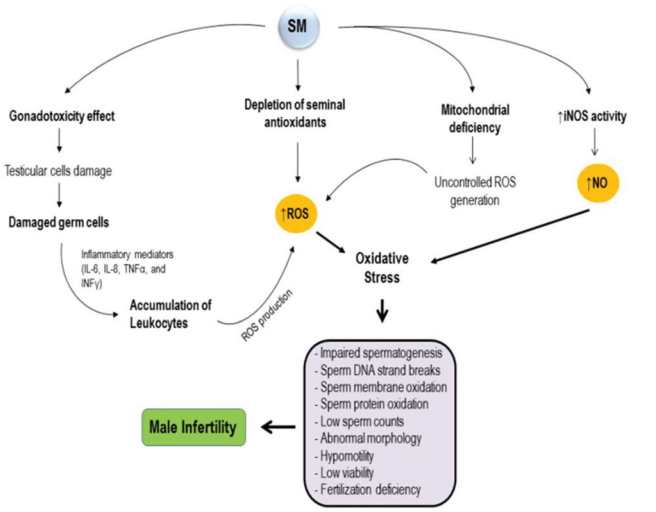

Fig. 4 Mechanisms through which SM induces oxidative stress and male infertility.

tion, its transport activity, and, eventually, spermatozoa survival (Fig. 4). Lipid peroxidation also has a deleterious effect on the ultramorphological structure of sperm cells and, therefore, on the male fertilization potential.[61] The oxidation of sperm membrane lipid axonemal proteins can be associated with permanent impairment of sperm motility because excessive ROS deplete cellular ATP, resulting in decreased phosphorylation of axonemal proteins, transient impairment of motility, and decreased sperm viability.[58]

Numerous studies have also shown that ROS can target sperm DNA by causing base modification, DNA strand breaks, DNA fragmentation and deletion, mutation, and chromatin cross-linking.[61–65] DNA damage can increase germ cell apoptosis and reduce sperm counts[66] (Fig. 4).

To counteract the toxic effects of ROS, human seminal plasma and spermatozoa are equipped with enzymatic and non-enzymatic antioxidants that act as ROS scavengers to protect sperm cells from oxidative damage.[58] The seminal plasma antioxidants are important because they compensate the depletion of sperm cytoplasmic enzymes when the cytoplasm is extruded during maturation.[67] However, overproduction of ROS in reproductive organs can overwhelm the effective contents of antioxidants, increasing the harmful effects of ROS on spermatozoa that are associated with abnormal sperm parameters.[68] SM can lead to excessive production of ROS, causing progressive oxidative damage and ultimately sperm cell death.

Role of SM in oxidative stress and inflammation

OS induced by free radicals is now believed to be a main mechanism of SM toxicity.[69,70] SM increases ROS production and OS through several mechanisms, including leukocyte accumulation and inflammation, reduced antioxidant activity, enhanced expression of ROS-production-related enzymes, mitochondrial dysfunction, depletion of glutathione (GSH) and GSH-dependent antioxidant enzyme productivity, and changes in the activity of inducible nitric oxide synthase (iNOS)[71] (Fig. 4).

A growing number of studies have confirmed a close relationship between the presence of leukocytes in semen and male infertility.[63] Some studies have shown that elevated levels of seminal ROS, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) are associated with increased sperm membrane lipid peroxidation and poor sperm quality.[72–74] Recent studies have shown that SM exposure is significantly associated with inflammatory reactions and oxidative injury at the site of damaged tissues.[70,75,76] Experimental studies have shown that SM can induce the secretion of several proinflammatory cytokines and growth factors, such as TNFα, IL-α, IL-β, IL-6, IL-8, IL-13, IL-15, and INF-γ, in damaged tissues.[77–80] SM can also accumulate several inflammatory cells, such as macrophages and neutrophils, with subsequent release of inflammatory mediators that can recruit and activate other leukocytes in reproductive system.[14] Activated leukocytes generate high levels of ROS that overwhelm the antioxidant defence systems, leading to increased OS in seminal plasma. Overproduction of ROS by SM-activated leukocytes causes oxidative damage to sperm DNA, protein, and membrane PUFA, which are associated with further inflammation, impaired spermatogenesis, apoptosis, and poor sperm quality[81] (Fig. 4).

Several studies have shown that SM induces mitochondrial dysfunction, which may be associated with electron transport chain deficiency, mass ROS production, DNA oxidation, and intracellular antioxidant depletion.[69,82] Spermatozoa are rich in mitochondria because a constant supply of ATP is necessary for their motility. An increased number of abnormal or immature spermatozoa significantly enhances ROS generation, which affects their mitochondrial function and, subsequently, sperm motility.[58,83]

SM can also impair spermatogenesis and induce sperm DNA fragmentation. In a previous study, the relationship between SM exposure and sperm DNA fragmentation was considered two decades after SM exposure.[81] A significant increase in the sperm DNA fragmentation index was found in SM patients, indicating an increased risk of congenital abnormalities and genetic defects in the offspring of SM-exposed victims created by assisted reproductive techniques (ARTs).[22,81]

SM can also reduce the effective concentration of antioxidants by enhancing ROS generation (Fig. 4). Glutathione (GSH) is a primary target for SM because its level is markedly reduced after SM exposure.[80] SM-GSH metabolites decrease cellular GSH and increase intracellular ROS and OS markers, including DNA, lipid, and protein oxidation.[80] Recent studies have shown that GSH and *N*-acetylcysteine (as a GSH prodrug) treatments reduce the OS and toxicity induced by SM.[84–86] SM can also decrease the activity of other antioxidants, such as thioredoxin reductase, catalases (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPX), and glutathione-*S*-transferases (GST), which are critical in controlling cellular antioxidant balance.[79,87] The reduced activity of these antioxidants can occur as a result of SM-induced alkylation or changes in the expression of these enzymes.

NADPH cytochrome p450 reductase, which plays a critical role in the detoxification of different toxic metabolites, is another target for SM.[88] Several studies have shown that SM not only inhibits the reduction of cytochrome C, but also prevents NADPH cytochrome p450 reductase activity and stimulates ROS generation.[88]

Conclusions

SM induces a wide variety of structural and functional disorders in the reproductive system, including reproductive hormone deficiencies, testicular cell damages, sexual dysfunction, spermatogenesis deficiency, poor sperm quality, and reduced fertility. OS is a major mechanism of SM action on human reproductive health. SM induces DNA fragmentation, lipid and protein oxidation, and, consequently, sperm apopto-

sis. SM induces OS in the reproductive system through several mechanisms, including the accumulation of leukocytes and inflammatory mediators, mitochondrial deficiency, enhanced activity of ROS-producing enzymes, reduced activity of intracellular antioxidants, GSH depletion and decreased productivity of GSH-dependent antioxidants, and, consequently, an imbalance between the production and detoxification of ROS in cells. Therefore, antioxidant therapy might help protect reproductive function against SM-induced damage.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

We are deeply indebted to past and present collaborators.

Notes and references

1 S. Namazi, H. Niknahad and H. Razmkhah, *J. Med. Toxicol.*, 2009, **5**, 191–195.
2 K. Kehe and L. Szinicz, *Toxicology*, 2005, **214**, 198–209.
3 Z. M. Hassan, M. Ebtekar, M. Ghanei, M. Taghikhani, M. R. Noori Daloii and T. Ghazanfari, *Iran. J. Allergy, Asthma Immunol.*, 2006, **5**, 101–108.
4 M. Balali, *Arch. Belg.*, 1984, (Suppl), 254–259.
5 R. Vijayaraghavan, *Arch. Toxicol.*, 1997, **71**, 157–164.
6 S. Khateri, M. Ghanei, S. Keshavarz, M. Soroush and D. Haines, *J. Occup. Environ. Med.*, 2003, **45**, 1136–1143.
7 M. Rowell, K. Kehe, F. Balszuweit and H. Thiermann, *Toxicology*, 2009, **263**, 9–11.
8 M. Ghanei and A. A. Harandi, *Inhalation Toxicol.*, 2011, **23**, 363–371.
9 K. Ghabili, P. S. Agutter, M. Ghanei, K. Ansarin and M. M. Shoja, *JAT, J. Appl. Toxicol.*, 2010, **30**, 627–643.
10 M. Shohrati, M. Peyman, A. Peyman, M. Davoudi and M. Ghanei, *Cutaneous Ocul. Toxicol.*, 2007, **26**, 73–81.
11 M. Ghanei, M. Rajaee, S. Khateri, F. Alaeddini and D. Haines, *Reprod. Toxicol.*, 2004, **18**, 635–639.
12 M. E. Soroush, M. R. Soroush and S. H. Khateri, *Iran. Red Crescent Med. J.*, 2008, **10**, 344–345.
13 S. Shakeri, M. Yazdani and E. Kheradpezhouh, *Iran. Red Crescent Med. J.*, 2007, **9**, 59–62.
14 E. Tahmasbpour Marzony, M. Ghanei and Y. Panahi, *Asian Pac. J. Reprod.*, 2016, **5**, 1–9.
15 P. Jost, H. Svobodova and R. Stetina, *Chem.–Biol. Interact.*, 2015, **237**, 31–37.
16 A. Najafi, A. Masoudi-Nejad, A. A. Imani Fooladi, M. Ghanei and M. R. Nourani, *J. Recept. Signal Transduction Res.*, 2014, **34**, 283–289.
17 D. R. Gerecke, M. Chen, S. S. Isukapalli, M. K. Gordon, Y. C. Chang, W. Tong, I. P. Androulakis and P. G. Georgopoulos, *Toxicol. Appl. Pharmacol.*, 2009, **234**, 156–165.
18 P. A. Jowsey, F. M. Williams and P. G. Blain, *Toxicol. Lett.*, 2012, **209**, 1–10.
19 M. P. Abasalt Hosseinzadeh Colagar, E. T. Marzony and S. G. A. Jorsaraei, *Braz. Arch. Biol. Technol.*, 2009, **52**, 1387–1392.
20 F. Azizi, A. Keshavarz, F. Roshanzamir and M. Nafarabadi, *Med. War*, 1995, **11**, 34–44.
21 M. A. Amirzargar, M. Yavangi, M. Rahnavardi, M. Jafari and M. Mohseni, *Int. J. Androl.*, 2009, **32**, 411–416.
22 M. R. Safarinejad, A. A. Kolahi and S. Iravani, *BJU Int.*, 2010, **105**, 79–86.
23 M. R. Safarinejad, *Urology*, 2001, **58**, 90–94.
24 H. Pour-Jafari and A. A. Moushtaghi, *Vet. Hum. Toxicol.*, 1992, **34**, 547.
25 A. A. Ketabchi, *J. Kerman Univ. Med. Sci.*, 1998, **5**, 74–79.
26 M. Balali-Mood, M. Hefazi, M. Mahmoudi, E. Jalali, D. Attaran, M. Maleki, M. E. Razavi, G. Zare, A. Tabatabaee and M. R. Jaafari, *Fundam. Clin. Pharmacol.*, 2005, **19**, 713–721.
27 Y. Panahi, Y. Moharamzad, F. Beiraghdar and M. M. Naghizadeh, *Basic Clin. Pharmacol. Toxicol.*, 2009, **104**, 171–175.
28 A. Z. Ghanei M, *J. Med. Chem.*, 2003, **1**, 1–9.
29 L. B. Sasser, J. A. Cushing and J. C. Dacre, *JAT, J. Appl. Toxicol.*, 1993, **13**, 359–368.
30 M. Balali-Mood, S. Mousavi and B. Balali-Mood, *Emerg. Health Threats J.*, 2008, **1**, e7.
31 K. Matsuo, S. Kooshesh, M. Dinc, C. C. Sun, T. Kimura and A. A. Baschat, *Am. J. Perinatol.*, 2007, **24**, 257–266.
32 Y. Panahi, M. Ghanei, K. Ghabili, K. Ansarin, S. Aslanabadi, Z. Poursaleh, S. E. Golzari, J. Etemadi, M. Khalili and M. M. Shoja, *J. Urol.*, 2013, **10**, 837–846.
33 M. Y. S. Shakeri and E. Kheradpezhouh, *Iran. Red Crescent Med. J.*, 2007, **9**, 59–62.
34 F. Azizi, H. Elyasi, H. Sohrabpour, N. Jalali and M. Nafarabadi, *Med. J. Islam. Repub. Iran*, 1989, **3**, 105–107.
35 K. Ghabili, M. M. Shoja, S. E. Golzari and K. Ansarin, *Curr. Urol.*, 2012, **6**, 112.
36 S. F. Agin K, *Int. J. Endocrinol. Metab.*, 2006, **4**, 130–135.
37 A. D. Nascimento, E. de Lima, G. Boechat, S. Meyrelles, N. Bissoli, D. Lenz, D. Endringer and T. de Andrade, *Hum. Exp. Toxicol.*, 2015, **34**, 1139–1147.
38 E. Tahmasbpour, S. Reza Emami, M. Ghanei and Y. Panahi, *Inhalation Toxicol.*, 2015, **27**, 659–672.
39 M. Hefazi, D. Attaran, M. Mahmoudi and M. Balali-Mood, *Inhalation Toxicol.*, 2005, **17**, 587–592.
40 S. Rao, A. Matsumura, J. Yoon and M. C. Simon, *J. Biol. Chem.*, 1999, **274**, 11115–11124.
41 A. Amir, S. Chapman, T. Kadar, Y. Gozes, R. Sahar and N. Allon, *JAT, J. Appl. Toxicol.*, 2000, **20**(Suppl 1), S51–S58.
42 C. L. Gross, H. L. Meier, B. Papirmeister, F. B. Brinkley and J. B. Johnson, *Toxicol. Appl. Pharmacol.*, 1985, **81**, 85–90.
43 B. Papirmeister, C. L. Gross, H. L. Meier, J. P. Petrali and J. B. Johnson, *Fundam. Appl. Toxicol.*, 1985, **5**, S134–S149.

44 A. M. Casillas, S. G. Clyman, Y. V. Fan and R. H. Stevens, *Adv. Health Sci. Educ. Theory Pract.*, 2000, **5**, 23–41.
45 C. M. Simbulan-Rosenthal, R. Ray, B. Benton, E. Soeda, A. Daher, D. Anderson, W. J. Smith and D. S. Rosenthal, *Toxicology*, 2006, **227**, 21–35.
46 P. Nicotera, G. Bellomo and S. Orrenius, *Annu. Rev. Pharmacol. Toxicol.*, 1992, **32**, 449–470.
47 J. G. Pounds, *Environ. Health Perspect.*, 1990, **84**, 7–15.
48 S. Orrenius, D. J. McConkey, G. Bellomo and P. Nicotera, *Trends Pharmacol. Sci.*, 1989, **10**, 281–285.
49 G. Pirzad, M. Jafari, S. Tavana, H. Sadrayee, S. Ghavami, A. Shajiei and M. Ghanei, *J. Toxicol.*, 2010, **2010**, 373612.
50 G. D. Minsavage and J. F. Dillman 3rd, *J. Pharmacol. Exp. Ther.*, 2007, **321**, 202–212.
51 M. Gomez-Lazaro, F. J. Fernandez-Gomez and J. Jordan, *J. Physiol. Biochem.*, 2004, **60**, 287–307.
52 A. H. Colagar and E. T. Marzony, *J. Clin. Biochem. Nutr.*, 2009, **45**, 144–149.
53 A. H. Colagar, E. T. Marzony and M. J. Chaichi, *Nutr. Res.*, 2009, **29**, 82–88.
54 A. Hosseinzadeh Colagar, P. Mehdi, E. Tahmasbpour Marzony and S. G. A. Jorsaraee, *Braz. Arch. Biol. Technol.*, 2009, **52**, 1387–1392.
55 A. Agarwal and T. M. Said, *BJU Int.*, 2005, **95**, 503–507.
56 R. J. Aitken, M. A. Baker and D. Sawyer, *Reprod. BioMed. Online*, 2003, **7**, 65–70.
57 A. H. Colagar, G. A. Jorsaraee and E. T. Marzony, *Pak. J. Biol. Sci.*, 2007, **10**, 3870–3874.
58 A. Agarwal, G. Virk, C. Ong and S. S. du Plessis, *World J. Men's Health*, 2014, **32**, 1–17.
59 D. Sanocka and M. Kurpisz, *Reprod. Biol. Endocrinol.*, 2004, **2**, 12.
60 K. Makker, A. Agarwal and R. Sharma, *Indian J. Med. Res.*, 2009, **129**, 357–367.
61 E. Tahmasbpour, D. Balasubramanian and A. Agarwal, *J. Assist. Reprod. Gen.*, 2014, **31**, 1115–1137.
62 N. Zribi, N. F. Chakroun, H. Elleuch, F. B. Abdallah, A. S. Ben Hamida, J. Gargouri, F. Fakhfakh and L. A. Keskes, *Reprod. Biol. Endocrinol.*, 2011, **9**, 47.
63 R. J. Aitken, G. N. De Iuliis, J. M. Finnie, A. Hedges and R. I. McLachlan, *Hum. Reprod.*, 2010, **25**, 2415–2426.
64 J. Bellver, M. Meseguer, L. Muriel, S. Garcia-Herrero, M. A. Barreto, A. L. Garda, J. Remohi, A. Pellicer and N. Garrido, *Hum. Reprod.*, 2010, **25**, 1713–1721.
65 C. Wright, S. Milne and H. Leeson, *Reprod. BioMed. Online*, 2014, **28**, 684–703.
66 N. P. Singh, C. H. Muller and R. E. Berger, *Fertil. Steril.*, 2003, **80**, 1420–1430.
67 A. Agarwal and S. A. Prabakaran, *Indian J. Exp. Biol.*, 2005, **43**, 963–974.
68 A. Agarwal, S. A. Prabakaran and T. M. Said, *J. Androl.*, 2005, **26**, 654–660.
69 D. Kumar, N. Tewari-Singh, C. Agarwal, A. K. Jain, S. Inturi, R. Kant, C. W. White and R. Agarwal, *Toxicol. Lett.*, 2015, **235**, 161–171.
70 M. Pohanka, R. Stetina, H. Svobodova, B. Ruttkay-Nedecky, M. Jilkova, J. Sochor, J. Sobotka, V. Adam and R. Kizek, *Drug Chem. Toxicol.*, 2013, **36**, 270–276.
71 I. Layali, A. Shahriary, A. Rahmani, N. Talatappe, E. Tahmasbpour, H. Rostami and A. Beigi Harchegani, *Immunopharmacol. Immunotoxicol.*, 2018, **20**, 1–7.
72 G. Lavranos, M. Balla, A. Tzortzopoulou, V. Syriou and R. Angelopoulou, *Reprod. Toxicol.*, 2012, **34**, 298–307.
73 J. C. Lu, Y. F. Huang and N. Q. Lu, *Zhonghua Nankexue*, 2010, **16**, 867–871.
74 K. C. Nandipati, F. F. Pasqualotto, A. J. Thomas Jr. and A. Agarwal, *Andrologia*, 2005, **37**, 131–134.
75 N. Tewari-Singh, A. K. Jain, S. Inturi, C. Agarwal, C. W. White and R. Agarwal, *PLoS One*, 2012, **7**, e46149.
76 M. Pohanka, *Mini-Rev. Med. Chem.*, 2012, **12**, 742–748.
77 Y. Panahi, S. M. Davoudi, F. Beiraghdar, M. Amiri, A. Saadat, E. T. Marzony, M. M. Naghizadeh and A. Sahebkar, *Skinmed*, 2013, **11**, 205–209.
78 I. Khaheshi, S. Keshavarz, A. A. Imani Fooladi, M. Ebrahimi, S. Yazdani, Y. Panahi, M. Shohrati and M. R. Nourani, *BMC Dermatol.*, 2011, **11**, 2.
79 M. Shohrati, A. Amini-Harandi, B. Najafian, A. Saburi and M. Ghanei, *Iran. J. Med. Sci.*, 2014, **39**, 382–386.
80 M. Jafari and M. Ghanei, *Clin. Toxicol.*, 2010, **48**, 184–192.
81 S. MR, *Curr. Urol.*, 2010, **4**, 71–80.
82 A. A. Brimfield, S. D. Soni, K. A. Trimmer, M. A. Zottola, R. E. Sweeney and J. S. Graham, *Free Radical Biol. Med.*, 2012, **52**, 811–817.
83 R. R. Henkel, *Asian J. Androl.*, 2011, **13**, 43–52.
84 M. Shohrati, I. Karimzadeh, A. Saburi, H. Khalili and M. Ghanei, *Inhalation Toxicol.*, 2014, **26**, 507–523.
85 M. Ghanei, M. Shohrati, M. Jafari, S. Ghaderi, F. Alaeddini and J. Aslani, *Basic Clin. Pharmacol. Toxicol.*, 2008, **103**, 428–432.
86 M. Shohrati, J. Aslani, M. Eshraghi, F. Alaedini and M. Ghanei, *Respir. Med.*, 2008, **102**, 443–448.
87 L. Mirbagheri, M. Habibi Roudkenar, A. A. Imani Fooladi, M. Ghanei and M. R. Nourani, *Iran. J. Allergy, Asthma Immunol.*, 2013, **12**, 153–160.
88 J. P. Gray, V. Mishin, D. E. Heck, D. L. Laskin and J. D. Laskin, *Toxicol. Appl. Pharmacol.*, 2010, **247**, 76–82.
89 F. Azizi, H. Elyasi, H. Sohrabpour, N. Jalali and M. Nafarabadi, *Med. J. Islam. Repub. Iran*, 1989, **3**, 105–107.
90 L. Kooshesh, H. Dashtnavard, H. Bahadoran, A. Karimi, M. Jafari and M. H. Asadi, *J. Iran. Anat. Sci.*, 2007, **5**, 27–36.