Arch Toxicol
DOI 10.1007/s00204-016-1911-8

REVIEW ARTICLE

An evidence-based review of the genotoxic and reproductive effects of sulfur mustard

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Received: 25 November 2016 / Accepted: 7 December 2016

Abstract Sulfur mustard (SM) is a chemical warfare agent which is cytotoxic in nature, and at the molecular level, SM acts as DNA alkylating agent leading to genotoxic and reproductive effects. Mostly, the exposed areas of the body are the main targets for SM; however, it also adversely affects various tissues of the body and ultimately exhibits long-term complications including genotoxic and reproductive effects, even in the next generations. The effect of SM on reproductive system is the reason behind male infertility. The chronic genotoxic and reproductive complications of SM have been observed in the next generation, such as reproductive hormones disturbances, testicular atrophy, deficiency of sperm cells, retarded growth of sperm and male infertility. SM exerts toxic effects through various mechanisms causing reproductive dysfunction. The key mechanisms include DNA alkylation, production of reactive oxygen species (ROS) and nicotinamide adenine dinucleotide (NAD) depletion. However, the exact molecular mechanism of such long-term effects of SM is still unclear. In general, DNA damage, cell death and defects in the cell membrane are frequently observed in SM-exposed individuals. SM can activate various cellular and molecular mechanisms related to oxidative stress (OS) and inflammatory responses throughout the reproductive system, which can cause decreased spermatogenesis and impaired sperm quality via damage to tissue function and structure. Moreover, the toxic effects of SM on the reproductive system as well as the occurrence of male infertility among exposed war troopers in the late exposure phase is still uncertain. The chronic effects of SM exposure in parents can cause congenital defects in their children. In this review, we aimed to investigate chronic genotoxic and reproductive effects of SM and their molecular mechanisms in the next generations.

Keywords Sulfur mustard (SM) · Genotoxicity · Infertility

Introduction

SM is a chemical warfare agent showing both acute and chronic pathological effects in humans upon exposure. There are various body organs affected by SM, which mainly includes skin, eyes and lungs (Ghabili et al. 2011). During the Iraq–Iran war (1983–1988), this agent was used many times by Iraqi military forces against both Iranian soldiers and Iraqi Kurdish residence. It has been reported that during this war, almost 100,000 Iranians suffered from the deleterious effects of SM (Emad and Rezaian 1997). Due to the SM attack, many death cases were reported in the Iranian population. The victims of such attacks were intensively investigated by Iranian researchers. They observed several complications among the victims of SM attacks. Respiratory, dermatological, hematological and endocrine complications were most commonly observed among SM victims. These chronic complications adversely affected the quality of life of exposed patients (Mansour Razavi et al. 2012). The use of SM, as a chemical warfare

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Published online: 28 December 2016

agent during the times of Iraq–Iran war and against Kurdish community, has been acknowledged by the United Nations Security Council (UNSC) (Council 1988). Presently, about 50,000 individuals are suffering from the chronic effects of SM, and they are seeking for efficient treatment measures to overcome these impediments (Emad and Rezaian 1997). The chronic complications may still arise after the exposure of individuals to SM, even after 20 years. This has been confirmed by a survey conducted on 34,000 ex-soldiers of the Iraq–Iran war. It has been investigated that the occurrence of chronic lungs, visual and cutaneous complications was 42.5, 39.3 and 24.5%, respectively (Razavi et al. 2013). Due to these chronic pathological complications, the routine activities and overall life style of patients were badly affected, leading to the impairment of general well-being features of life (Panahi et al. 2008; Ebadi et al. 2014). Apart from some development in the area of the therapeutic measures for the better treatment of chronic complications that arose due to exposure of SM, there is still limited management plan available to overcome these difficulties. The reason behind this limitation is the lack of detailed much evidence regarding the molecular mechanism of mustard gas intoxications and the pathways of metabolism, which are mostly disrupted due to exposure of SM (Poursaleh et al. 2012; Zamani et al. 2016).

SM metabolites in the blood and/or other tissues detection are the potential biomarker for the evidence in the warfare troopers. So, metabolomics is a new branch of omics, which can determine various types of metabolites present in the individuals body sample after extensive exposure to warfare agents. The key point of metabolomics is specially applied for the purpose of diagnosing specific disease due to the ability of this advanced technology to accurately detect the reasons, which represent the external changes. The metabolomics experiments are carried out through either the use of mass spectrometry (MS) or nuclear magnetic resonance (NMR) techniques. These analytical techniques are useful for the identification of metabolites in those veterans affected by mustard gas attacks that can also provide the spreading mechanisms of toxicity related to mustard and help in the development of nominal therapies (Spratlin et al. 2009).

SM directly affects the skin, eyes and reproductive system, which consequently spread to the other body organs, and hence, the whole physiological system is disturbed (Ghabili et al. 2010). Besides the acute attacks of SM, there are prevalent chronic effects on different body organs like eyes, skin and immune system. Moreover, in mild cases, these effects also develop in the gastrointestinal system, cardiovascular and nervous systems (Balali-Mood et al. 2008).

The adverse and carcinogenic effects related to exposure of SM have been investigated in laboratory animals and on human models (Zafarghandi et al. 2013). SM is lipophilic in nature, and due to the presence of this lipophilic property, it can easily penetrate in the human body and cause DNA impairments. Due to this DNA damage, the chronic toxic effects of SM develop in the body with subsequent alteration in overall body function (Batal et al. 2014; Razavi et al. 2016). It is evident that SM potentiates OS either through the increased production of ROS from endogenous substances or causes decrease in antioxidant capacity and DNA repair capabilities (Jost et al. 2015). Due to the OS, DNA damage occurs, which in turn results in chromosomal instability and mutation in genes causing cell death (Najafi et al. 2014).

The reproductive system is one of the major targets of SM chronic toxicity in exposed individuals. Occurrences of infertility ratio among SM-exposed patients have been reported from 2.5 to 35% (Ghanei 2004; Soroush and Modirian 2008). After exposure to SM, there is a marked increase in the follicle-stimulating hormone (FSH), while the level of testosterone hormone decreases along with lessened semen quality (Azizi et al. 1995; Panahi et al. 2013). Due to SM exposure, the rate of fetal death and alteration in sex ratio has been reported in offspring of Iranian war veterans (Soroush and Modirian 2008). Various studies illustrated that SM has harmful effects on the reproductive system of both male and female, but the mechanism through which SM produces these effects is still unknown.

It is clear from recent studies that chronic genotoxic and reproductive effects of SM are primarily due to its ability to form mutations on gene level (Jowsey et al. 2012). It has been reported that SM potentiates OS through increased production of ROS, which in turn damage DNA, resulting in chromosomal aberration and cellular growth variation that ultimately result in cell death (Najafi et al. 2014). Therefore, the noxious effects of SM on various body cells are possibly the result of DNA damage initiated by alkylation of intracellular components or due to the ROS production and OS (Ghanei and Harandi 2016). Though, SM has been confirmed as a carcinogenic agent through laboratory experiments, and its genotoxic effects, especially in chronic situations, have not been addressed yet. The purpose of this review was to focus on the chronic genotoxic and reproductive effects of SM in the next generation.

Methods

The scientific information gathered in this review was collected by a widespread search of several electronic databases, including PubMed, Scopus, Medline, Web of Science, Embase and Google Scholar. The criteria for the exclusion of articles were the language of reports being

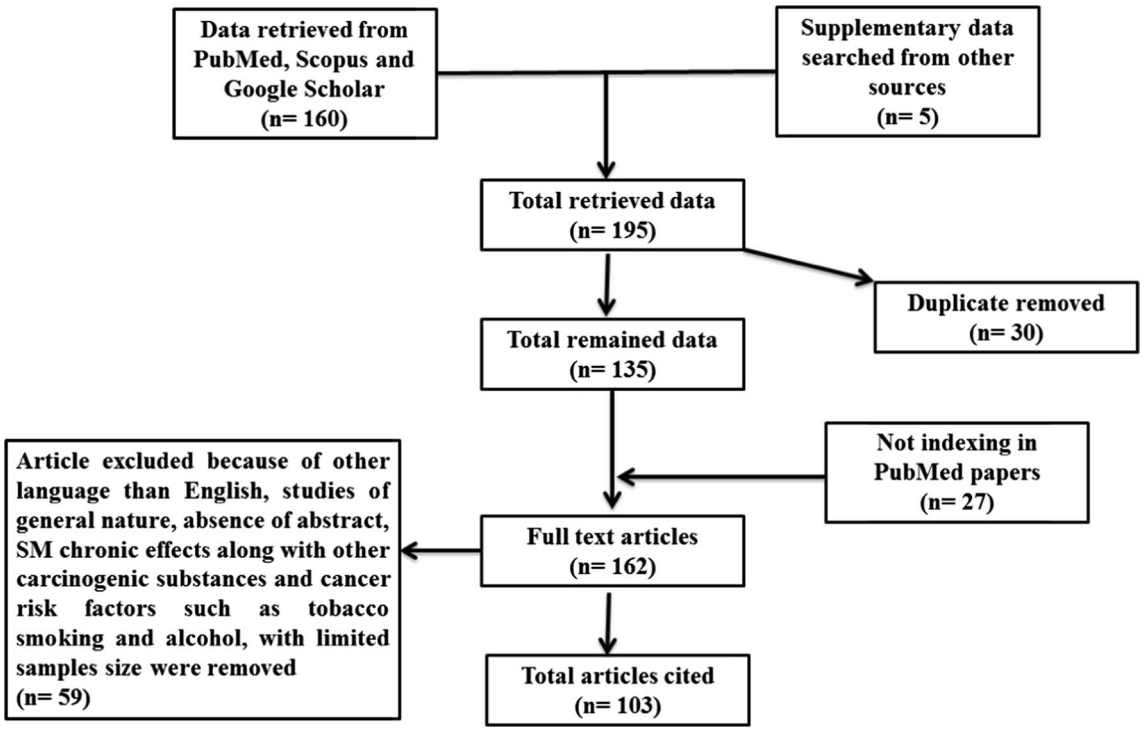

Fig. 1 Flow diagram of included studies. The number of citations and resource materials that have been screened, excluded and/or included in this review is indicated in *parenthesis*

other than English, reports with unavailable abstracts and studies related to SM acute and chronic effects apart from genotoxic and reproductive effects, which showed the linkage between cancer and cancer risk factors, such as tobacco smoking and alcohol consumption. Various appropriate articles not indexed by PubMed were also considered, and 27 such reports which fulfilled the criteria for inclusion were further recovered from Google Scholar and Google. Therefore, the total numbers (*n*) of included articles in this review were 103 (Fig. 1).

Physiochemical properties of SM

SM is pale yellow in color. It is an oily liquid with a molecular weight of 159.08 g mol^{-1}, having specific gravity 1.27 g mL^{-1}; viscosity is 0.046 poise and refractive index 1.531. The freezing point of SM is 14.4 °C. The vaporization temperature of SM is 25 °C. The odor of SM is similar to garlic. It is dangerous for human health both in vapor and in liquid forms. SM is poorly soluble in water, but it is readily soluble in organic solvents such as ether, acetone and other organic hydrocarbons (OHCs). It is especially soluble in rubber and food products (Feister 1991; Davis and Aspera 2001). SM has a characteristic smell closely similar to onions. Additionally, SM can damage the respiratory system, even in very low concentration. It decomposes in water, producing non-poisonous products through hydrolysis. The reaction of SM in aqueous solution is catalyzed by alkali. The reaction of SM with bleaching powder and chloramine is very violent, forming non-poisonous products (Davis and Aspera 2001).

Genotoxic effects of SM

The genome of the human body is composed of DNA, which is a characteristic genetic macromolecule found inside the nucleus. This DNA is mostly responsible for the transmission of characters from parents to offspring, and the transfer of such genetic information is important for human life (Sauvaigo et al. 2016). The chemical structure and composition of DNA are such that it is mostly vulnerable to changes upon exposure to chemical warfare agents such as SM. After exposure, there is an alteration in the three-dimensional structure of DNA and capacity to perform its role also changes (Jackson and Bartek 2009). The genomic properties of the cells often change, when it has been exposed to certain chemical agents that ultimately transform the bases of nucleotides and finally break the backbone of sugar phosphate, leading to mutation (Kastan and Bartek 2004).

There are physical and chemical agents that alter the genomic structure of the cells by changing the pattern of nucleotides bases or through breaking the central sugar-phosphate linkage. The agents, which directly affect and damage DNA, are either endogenous substance such as the by-products produced as a result of inflammation and/or it may be exogenous substances, which are present in water, food and environment. These chemical agents cause DNA damage by producing ROS leading to the cancer development (Kastan and Bartek 2004).

SM is an alkylating agent that produces their genotoxic effects by covalently transferring alkyl group to the DNA bases (Helleday et al. 2008). After interacting with intracellular molecules such as proteins and nucleic acids, it causes DNA damage and forms DNA adducts. Mostly, the alkylation of DNA consisting of adducts at guanine on *N*-7 position and at adenine on *N*-3 position constitutes total 61 and 16% of alkylation, respectively (Ludlum et al. 1994). The formation of di-DNA adducts represents almost about 15% of SM-induced DNA lesions inside the cell. In this reaction, one SM molecule reacts with *N*-7 positions. The di-adducts formation occurs inside the DNA strands producing intra-cross linking (Ludlum and Papirmeister 1986). In a study, the number of DNA single strand has been quantified in cultured human keratinocytes by using alkaline elution method. The introduction of DNA single-strand break was immediately measured after 30-min exposure of SM and was mostly dose-dependent (Szabo et al. 1996). The process of further DNA assimilation of strands breaks may be started within the process of DNA alkylation (Papirmeister et al. 1985).

The DNA-damaging properties of SM are important for its genotoxicity; the reaction of SM with other macromolecules within the cell should not be ignored. It was noted that cytoskeletal variations like damaging of stress fibers continued in endothelial cells after exposure to SM, although the nucleus was absent. Moreover, in comparison with endothelial cells, the endothelial materials did not exhibit the initial symbols of apoptosis such as the activation of caspase-3 and phosphatidylserine (Lodhi et al. 2001). Therefore, the alkylation of DNA seems to be mainly responsible for initiating the apoptosis, but not associated with the majority of the genotoxic effects of SM.

There are some available in vitro and in vivo studies that concentrate on DNA damage triggered by the application of SM. The evidence of DNA damage and fragmentation was observed in keratinocytes and thymocytes after exposure to SM (Heinrich et al. 2009). It has been reported in a study that SM causes DNA damage in mice models after inhalation through nasal or oral route. SM is responsible for the breaking of double strands of DNA in mice (Joseph et al. 2011). In a similar study conducted on lymphoid cells of mice, it has been investigated that SM is the main agent behind the intensity of DNA damage and this damage was mostly dose-dependent in nature (Jowsey et al. 2009).

The chemical agents responsible for the DNA damage are mostly produced from precursors in the form of a metabolic intermediate. These precursors are called as procarcinogens, although the intensity of carcinogenicity has not been exhibited in all the studied cases (Aydin et al. 2016). The procarcinogens include majority of the natural genotoxic products and other environmental toxicants, as they are the most important agents interacting with the DNA inside the cells and exert its genotoxic effects. The reaction of these toxicants is mostly indirect, as they interrelate with other molecules before interacting with the DNA, while the chemotherapeutic drugs act directly instead of interacting with DNA molecules (Nair et al. 2016).

The DNA-damaging properties of SM are crucial for the genotoxicity. However, the reaction of SM with other molecules should be considered as the possible threat of the DNA damage. It was observed that certain body changes such as cytoskeletal changes continue to the endothelial cells even after the treatment of SM. Apart from the endothelial cells, the cytoplasm of the endothelial cells did not show the possible symbols such as caspase-3 activation and the externalization of the phosphatidylserine; hence, it is clear that the alkylation of DNA is mostly responsible for the initiation of apoptosis, but merely all other cytotoxic effects caused due to SM (Nourani et al. 2016).

The reaction of the SM with the body cellular system such as antioxidants system is an interesting aspect of the possible toxic effects, which has been exhibited by the chemical warfare agent. There is a lot of available evidence that SM directly reacts with the intracellular thiols such as glutathione. After interacting with the cellular thiols, there is a marked depletion in the level of the intracellular glutathione. It has been reported in different cell culture and other in vivo studies (Pant and Lomash 2016).

Reproductive effects of SM

The adverse effects caused due to chronic exposure to SM on reproductive system have been addressed in very few studies in the past years. However, such studies showing the harmful effects of SM especially conducted on sperms quality and male infertility problems are increasing with time. There are several available investigative reports obtained from the studies conducted on animal models, suggesting that SM exposure leads to different structural and functional abnormalities in the male reproductive system causing endocrine disruption including an imbalance in reproductive hormones, damage to testis and finally sexual

Table 1 Chronic toxic effects of SM on male reproductive system

Study model	Duration	Toxic effects	References
SM victims	Several years	↓Infertility (23.3%); ↓decrease sperm quality (38.7%); ↑Abortion (13.6%); ↑sexual dysfunction (9%); ↓libido (30%); ↑premature ejaculation (23.6%); ↑FSH (57.6%); ↑LH (66.3%)	Pour-Jafari and Moushtaghi (1992)
SM victims	1st week after exposure	↓Free serum testosterone; ↓dehydroepiandrosterone (DHES)	Azizi et al. (1989)
SM victims	5th week after exposure	↓Free serum testosterone; ↓dehydroepiandrosterone (DHES)	Azizi et al. (1995)
SM victims	3rd and 5th week after exposure	↑Serum FSH: ↑serum LH	Safarinejad (2001)
SM victims	3 years after exposure	↓Free serum testosterone; ↑testicular atrophy; ↓spermatogenesis; ↑sertoli cell on pattern	Pour-Jafari and Moushtaghi (1992)
SM victims	4 years after exposure	↑Sperm counts;	Amirzargar et al. (2009)
SM victims	10 years after exposure	↑Abnormal sperm (38%); ↑abnormal morphology of sperm (54%); sperm motility (48%)	Shakeri and Yazdani (2007)
SM victims	15 years after exposure	↑Oligozoospermia (10%)	Ghanei et al. (2008)
SM victims	20 years after exposure	↓Semen volume; ↓sperm counts; ↓sperm motility; ↓normal morphology of sperm; ↑sperm DNA damages	Safarinejad (2010)
Male rats	10 days	↑Abnormal sperm; ↓sperm counts; ↓sperm motility	Sasser et al. (1993)
Male rats	10 days	↑Abnormal sperm, ↓sperm counts; ↓sperm mobility; ↓free serum testosterone; ↓testis weight	Kooshesh et al. (2007)

dysfunction. Apart from these adverse effects, SM exposure can cause genital lesions and impaired sperm quality and quantity (Panahi et al. 2013).

It is evident from several studies that exposure to SM causes poor sperm quality, and the spermatozoa are especially prone to the adverse and toxic effects (Rezvanfar et al. 2008). It has been reported that SM causes azoospermia and oligospermia in 42.5 and 57.5% cases, respectively, in individuals upon exposure (Safarinejad 2001). It has been described that SM exposure causes abnormality of sperm (53.8%), reduction in sperm motility (48.4%), decreased sperm count (23.1%) and abnormal semen viscosity (17.6%), as the frequently detected semen-associated abnormalities in patients previously exposed to SM (Panahi et al. 2013). In a study conducted on patients exposed to SM during Iran–Iraq warfare, the samples obtained from the semen of patients were analyzed to find out sperm abnormalities. The results obtained from this study indicated that sperm abnormalities and related disorders were found in 38% of the SM-exposed victims (Panahi et al. 2013). In a similar study, the chronic effects of SM on the testicular system and male fertility have been carried out 20 years after exposure. It has been confirmed in this study that male infertility was common in 23% patients after exposure to SM and the level of semen and quality was significantly decreased in such patients (Table 1) (Amirzargar et al. 2009).

Various studies have shown that SM toxicity disrupts the normal level of male sex hormones, which are very important for the regulation and maturation of the spermatogenesis process. Furthermore, it has been observed that SM inhibits hypothalamus–hypophysis–testis axis, mainly associated with reduced spermatogenesis and impaired sperm quality. Follicle-stimulating hormone (FSH) and luteinizing hormones (LH) are mostly responsible for the development of both germ cell and spermatogenesis. Hence, unusual spermatogenesis is associated with an impaired level of previously mentioned hormones especially testosterone. It has been reported in recent studies that the drastic changes in the level of gonadotropins and testosterone concentrations were mostly because of SM exposure (Azizi et al. 1989; Safarinejad 2001). A detailed study conducted by Azizi and co-workers confirmed that SM exposure causes a significant decrease in the level of androgen hormones' poor responsiveness to gonadotropic releasing hormone (GnRH). Similarly, a marked decreased level of free testosterone (FT) and dehydroepiandrosterone was observed after chronic exposure to SM (Agin and Sarvghadi 2006). Moreover, the sperm count was completely associated with the level of testosterone. When there is a marked decrease in testosterone concentration inside the testicular region, it seems to be a major motivator for apoptosis in overall germ cells, and ultimately, it will affect sperm and male fertility (Do-Nascimento et al. 2015). Thus, when there is a reduction in the level of testosterone hormones due to SM exposure, it is mostly predictable that it will alter the instigation of spermatogenesis, and ultimately, it will lead to a proliferation of the germ

cell along with low sperm quality. Moreover, other disorders such as decreased sperm count and related sperm abnormalities have shown to be mostly connected with a raised FSH level. As the level of FSH elevated, it is the main symbol of abnormal spermatogenesis and direct testicular function failure. It is suggested that decreased sperm count in SM-exposed individuals is mostly associated with testicular injury, which is a clear indication of SM gonadotoxicity (Amirzargar et al. 2009).

There are various studies conducted on biopsy samples obtained from the testis of SM-exposed individuals. It is evident that SM causes a complete arrest of spermatogenesis process, Sertoli cells as well as Leydig cells (Safarinejad et al. 2010). Therefore, decreased testosterone production due to SM exposure would possibly inhibit the initiation of spermatogenesis, and ultimately, it will lead to an excessive proliferation of the germ cell apoptosis and impaired sperm quality. Therefore, spermatogenesis is the key target of injury occurred in gonads as a result of SM exposure. Due to the inhibition of the spermatogenesis in the testis, the SM-exposed patients present many other adverse effects like decreased semen volume due to the obstruction of the duct responsible for ejaculation as well as impaired sperm quality. Sexual dysfunction is also reported in SM-prone patients. In a study conducted on 800 Iranian war veterans exposed to SM, it was observed that 35% of these patients exhibited decreased libido (Pour-Jafari and Moushtaghi 1992). In a similar study, erectile dysfunction and premature ejaculation were reported in 9 and 23.3%, respectively, in SM-exposed individuals (Ketabchi 1998). The above-mentioned complications are due to reduced level in testosterone. Other pathological problems such as genital lesions have been observed in SM-exposed patients (Balali-Mood et al. 2005; Panahi et al. 2009).

The chronic pathological effects of SM on the reproductive system and sperm quality have been investigated in animal models. It has been observed that elevated percentage of abnormal sperm and defects in spermatogenesis was noticed in male rats' models, which had been exposed to SM at the dose rate of 0.50 mg per kg (Kooshesh et al. 2007). In a similar study conducted on male rats model, variations in testicular tissue integrity and reduction in weight of testis after the injection of SM through intraperitoneal route have been observed (Matsuo et al. 2007).

Molecular mechanisms of SM genotoxicity

SM is lipophilic in nature; it can penetrate into the body and easily infiltrate to various organs such as eyes, skin and respiratory system (Balali-Mood et al. 2005). After absorption, SM readily distributes to all parts of the body systemically and adversely affects tissues and organs, especially the reproductive system, which leads to male fertility problems. 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 (Rao et al. 1999; Matsuo et al. 2007). Consequently, inflammatory responses such as the release of cytokines and tissue damage events began particularly in exposed area. Though SM causes alkylation of various intracellular molecules, DNA damage induced by SM exposure is the main motivator of overall cellular responses, which is responsible for cell injury and death (Amir et al. 2000a, b). SM makes structural changes in DNA, as it holds single alkylation point, which in turn attacks nitrogenous groups of DNA (Amir et al. 2000a, b). The toxic effects exhibited via SM have been accredited to DNA modification; hence, upon interaction of SM molecules with DNA, they not only cause the breaking of DNA strands and/or modification in DNA structure, but also bring modification in DNA replication and transcription process, leading to cell death (Jowsey et al. 2012). Moreover, SM has the ability to interact with protein molecules and inhibit the functioning through oxidation and denaturing of enzymes. SM is also responsible for the lipid peroxidation upon exposure, and after the process of peroxidation, free radicals are formed and finally released as by-products. It is believed that OS persuaded via the formation of free radicals is the main effect associated with SM toxicity and exposure followed by the inhibition of cell-signaling pathways and ultimately cell death (Ruff and Dillman 2010).

The depletion in NAD is an alternative mechanism responsible for cell damage. After the DNA damage and structural modification induced through SM, there are several other cellular pathways such as poly(ADP-ribose) polymerase (PARP) and nucleotide excision repair; these pathways are activated after exposure of individuals to SM. It has been revealed that as the strand of DNA breaks, it causes PARP activation, which in turn leads to NAD^+ depletion that enhanced synthesis of proteases; this increased production of proteases is linked with cellular injury and programmed cell death (Gross et al. 1985).

There are various available studies, which consider calmodulin and increased secretion of intracellular calcium ions Ca^{2+} level as prominent molecules triggered upon SM exposure (Ray et al. 1995). This increase in intracellular Ca^{2+} ions is due to protein kinase signaling pathways activation that leads to the activation of phospholipase C (PLC) and production of inositol triphosphate (IP3). These mediators act on Ca^{2+} stores inside the cell causing the release of the Ca^{2+} ions (Nicotera et al. 1992). There is another possible mechanism associated with increased influx of Ca^{2+} ions. This mechanism involves the production of ROS

caused by SM exposure. These ROS react with Ca^{2+} channels inside endoplasmic reticulum and cell membrane. The interaction of ROS with membrane causes an increased influx of Ca^{2+} ions into the cytosol. This elevated level of intracellular and cytosolic Ca^{2+} increases proteases activity and also triggers phospholipase activity, which ultimately degrades intracellular proteins along with DNA damage (Pounds 1990).

OS and male infertility due to SM

OS is the term used to describe the imbalance between the bioavailability of ROS and intracellular antioxidant production that lead to serious failure of the body functions and finally cause cell death (Colagar and Marzony 2009). These ROS or free radicals are produced as a result of metabolic reactions in the body of living organisms. These ROS are hydroxyl ion and superoxide ions (OH^-) and superoxide anion (O_2^-) (Colagar et al. 2009a, b). These free radicals are unstable in nature having short life span, which severely affects cellular reactions and alters the chemical structure of proteins and DNA molecules in a much higher concentration. However, normal sperm affectivity is most susceptible to even small concentration of ROS, and hence, the quality and quantity of sperms are affected, leading to male infertility (Colagar et al. 2009a, b). It has been confirmed that OS tempted via the production of ROS is the leading cause of reduced sperm quality, sperm dysfunction and male infertility (Fig. 2). Human spermatozoa are the most susceptible cell to OS (Agarwal et al. 2014).

It is now evident that OS initiated by the production of free radicals is a key mechanism through which SM exerts its toxic effects, especially on the reproductive system of the human body. It is clear that these ROS are mainly responsible for the production of OS, which in turn desperately affects the sperm quality and quantity, leading to male infertility. It has been observed that SM causes an increased production of ROS particularly in the testis and adversely alters the sperm chemical structure and function (Pohanka et al. 2013; Kumar et al. 2015). SM induces OS in reproductive system with various mechanisms. The increased production of ROS is one of the key mechanisms through which the male infertility problems are closely associated and exert a destructive effect on the normal cells of the reproductive system shown in Fig. 2 (Tahmasbpour et al. 2014).

It has been confirmed that there is a close correlation between male infertility and elevated level of ROS and tumor necrosis factor. It is also evident from this study that there is a linkage between the presence of leukocytes in male ejaculate and male factor as the basic reason of male infertility (Lu et al. 2010; Lavranos et al. 2012). It has been shown in various studies that SM exposure induces the increased production of inflammatory cytokines and human growth factors such as tumor necrosis factor alpha (TNFα), interleukins (IL), macrophages chemotactic proteins (MCP)-1 and matrix metalloproteinases (MMPs) in the injured cells and tissues illustrated in Fig. 3 (Khaheshi et al. 2011).

Physical abnormalities in the next generation due to SM exposure

It has been investigated in a study that majority of physical anomalies in children are mostly associated with the exposure of their fathers to SM. The frequency of such physical disorders is more common in war veterans, which have been previously affected due to SM attacks in the battlefield or in residential areas (Abolghasemi et al. 2010). Moreover, there was a close correlation between paternal exposure and the associated disorders appeared due to SM exposure such as respiratory complications along with congenital deformities (Watson et al. 2006). These defects are significantly more in war-torn regions and previously exposed localities of Iran such as Sardasht and Rabat during Iran–Iraq warfare. The inhabitants of these cities are adversely affected due to SM, and their newborn babies developed congenital defects such as microcephaly, ventricular septal defect and aortic stenosis (Abolghasemi et al. 2010). It has been confirmed in a study that both humans and common animals exposure to certain chemical warfare agent leads to potential abnormalities in their offspring (Bujan et al. 2013). It has been revealed in several studies that after exposure to cyclophosphamide, male rats and mice show congenital defects in their offspring (Jenkinson et al. 1987; Jenkinson and Aderson 1990). In human beings, paternal exposure to different toxic substances such as acrylamide and lead causes a significant increase in the development of congenital disorders in the newborn babies (Zhang et al. 1995). As we discussed previously that DNA damage is involved in the possible mechanism of SM toxicity, this damaged DNA and related genetic materials will be transferred from parents to offspring, and eventually, birth defects will arise in such children (Uzych 1985).

SM is genotoxic and cytotoxic in nature, causing mutagenicity and carcinogenic effects (Gross et al. 2006). The active metabolite of SM such as sulfonium ion readily reacts with intracellular proteins and nucleic acids and, eventually, changes the chemical structure of cellular contents. Such metabolites of SM are responsible for the alteration in the functional groups like OH^- and SH producing alkylation products. This process of alteration in the three-dimensional structure of the protein and nucleic acids

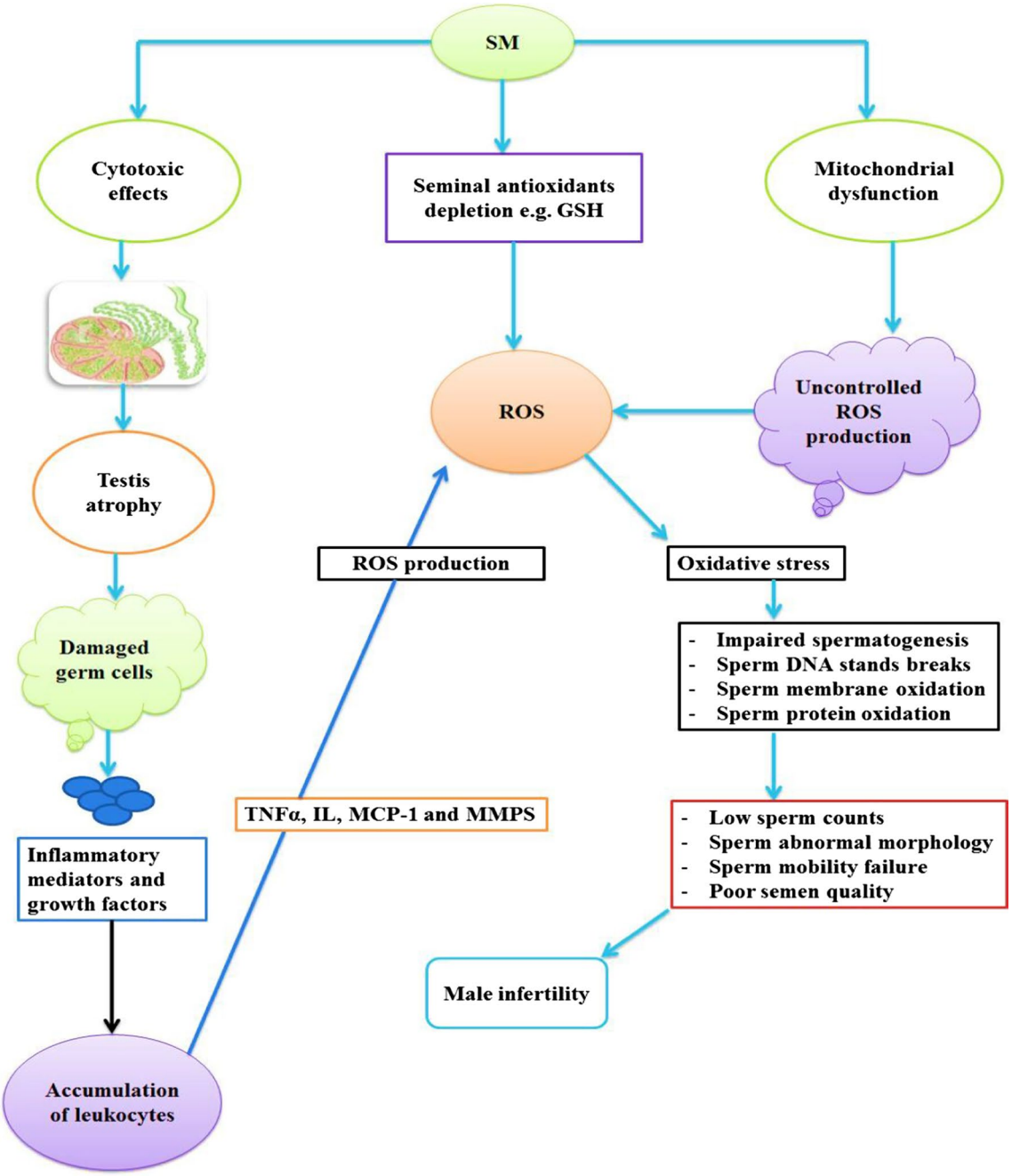

Fig. 2 Effect of ROS and OS induced by SM on sperm cells and male infertility

is tremendously toxic for the cells (Balali-Mood and Navaeian 1986).

It has been confirmed by several studies that SM exhibits its toxic effects on spermatogenesis, which in turn explains the possible elevation of physical anomalies among the newborn babies of the victims of this chemical warfare agent (Azizi et al. 1995; Karalliedde et al. 2000). It has been investigated in a study carried out on the Iranian war victims of the chemical warfare agents that frequency of the congenital defects among the children was 33% in the total reported cases (Pour-Jafari 1994). In a similar study, it has been observed that the number of cleft lip and cleft palate cases among children has increased in war victims, but they were unable to establish a correlation between these two particular events (Taher 1992).

It has been confirmed in a study that there is a close association between SM exposure and congenital defects. Although in this study they did not find particular abnormality, which

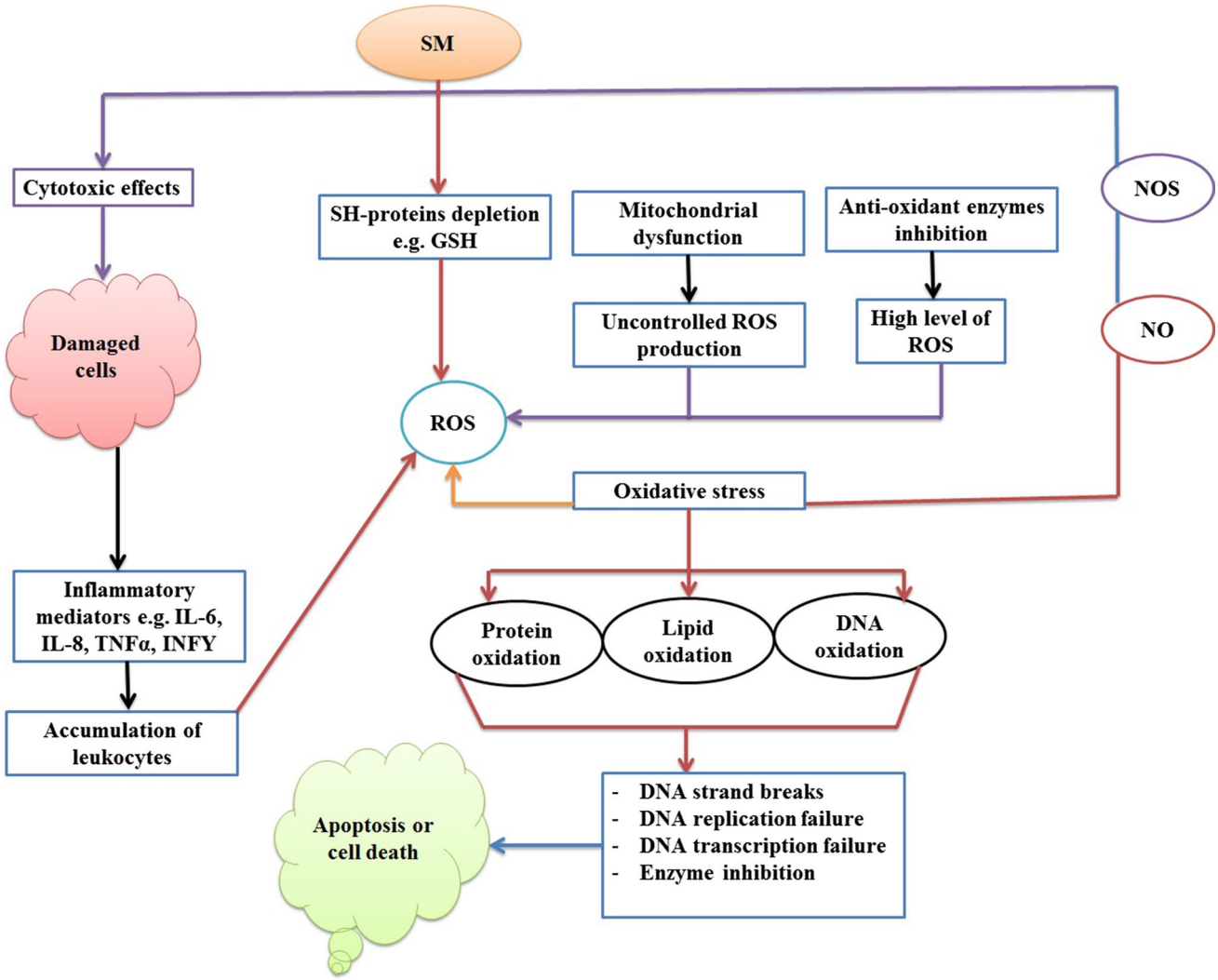

Fig. 3 Mechanisms through which SM induces OS and cell death

is associated with exposure to SM in that specific area except the case of anencephaly, the prevalence rate of that case was one among 1000 live births (Aguiar et al. 2003). It has been observed that there is a close association between the frequency of the physical anomalies and paternal exposure to SM. This study revealed that the use of SM in conflicts has a devastating effect on the children of both soldiers and civilians victims (Abolghasemi et al. 2010).

It has been observed in a study that SM exposure is the leading cause of physical abnormalities among the progenies of the Iranian population (Abolghasemi et al. 2010). This study is based on examinations and practical findings obtained from the war victims. During this study, several physical abnormalities have been investigated in the children of the war-affected individuals. All such abnormalities were coded according to the International Classification of Diseases, revision 10 (ICD-10). In this study, it was revealed that 283 couples had acute SM exposure (Nourani et al. 2016). In 193 couples, among the investigated population, only the father was a victim of the SM at least 9 months before conception. Also, 19 men were found primary infertile and 07 men were found with secondary infertility. Therefore, 164 couples were labeled as exposed group. Similarly, 136 couples were labeled as the non-exposed group (Abolghasemi et al. 2010).

Current treatment for SM genotoxicity

To develop a desirable treatment strategy for SM toxicity, there has been made considerable progress in the recent years. Among the long-term and serious effects of SM intoxication, respiratory disorders are the most serious observed effects. The complications of the respiratory effects are more

difficult to manage as compared to skin burns. Therefore, it is important to improve the research methodologies in order to identify modified pharmacological goals in terms of respiratory injuries (Graham et al. 2005). It has been revealed that steroids and non-steroidal anti-inflammatory drugs are useful for the toxicities of SM (Graham et al. 2005). Hence, it is important to get a comparatively good understanding about how to activate necrotic factor kappa B (NFκB) and the possible release of prostaglandin. In addition, the activation mechanism for MMP is available in every organ, which has been exposed to SM. Though, there are available MMP inhibitors with significant importance in preventing the lung damage (Guignabert et al. 2005). Apart from MMP inhibitors, *N*-acetylcysteine is an effective agent for the treatment of lesions developed in the pulmonary system after an individual has been exposed to SM via inhalation route. Therefore, it is necessary to gather more data regarding the significance of ROS and nitric oxide formation, which is responsible for the OS and inflammation within the body. The use of MMP is beneficial for lowering the magnitude of inflammation and tissue damage after the exposure of SM. Apart from these therapeutic plans for the treatment of SM toxicities, it is difficult to evaluate the consequences of pharmacological treatment of SM-induced cell death and damage to the DNA. It has been investigated that the apoptosis induced by SM in vitro can be inhibited by the inhibition of caspase (Simbulan-Rosenthal et al. 2006). In the case of necrosis, it would improve the inflammatory response drastically, and eventually, it will promote the tissue damage. In the case of survival process inside the cell, many cells have different intensity of DNA damage, which are more susceptible to mutagenic transformation. Therefore, it will be a good strategy to eradicate those cells, which have been damaged by SM as soon as possible, while in case of topical skin injury, the surgical removal of the wounds debris is preferred. This will, in turn, improve the wound-healing process (Graham et al. 2005). Moreover, the surgical procedure shall not be applicable for mass causalities. Also, lungs and eyes cannot be managed in this way; therefore, it is important to design a pharmacological approach. The combination of different drugs can reduce the inflammatory response, and it can avoid the damaged cells from survival.

There is an interesting drug family, which should be kept in consideration in this context, these drugs are the pharmacological inhibitors of PARP-1, having the ability to enhance apoptosis and necrosis (Rosenthal et al. 2001). These drugs can down-regulate various tissue and inflammatory pathways. The PARP-1 inhibitors have the ability to indirectly diminish OS by overwhelming the inflammatory response and also by inhibiting the infiltration of activated mononuclear cells (Jagtap and Szabó 2005). It has been indicated in a study that natural polyphenolic compounds have a significant effect in the prevention of OS and other inflammatory conditions (Jafari et al. 2014). It will be a good remedy to treat various cancer and inflammatory conditions caused due to SM with these natural polyphenols. The natural polyphenolic compounds have been considered as important secondary metabolites in the treatment of cancer. These are a large family of plant-based chemopreventive agents. Among natural phenolic compounds, the flavonoids are useful for the treatment of OS and cancer (Khan et al. 2016). There are plant-derived drugs such as resveratrol having a significant effect to treat OS and inflammation caused due to SM. It will be a potential candidate to treat such conditions linked with the toxicities of SM (Saeidnia and Abdollahi 2013). Regardless of the continuous efforts during the past years, still no specific antidote has been found to treat the SM toxicity. The current knowledge of drugs and their applications in the modern systems of medicines have discovered new area of treatment associated with chronic effects of chemical warfare agents (Kehe and Szinicz 2005). Similarly, to overcome the vesicant effects of SM, there are certain degradation products such as thiodiglycol. These environmental products are considered as significantly persistent. Therefore, it is important to involve the evaluation of both thiodiglycol and SM, especially in those areas, where there is potential contamination (Munro et al. 1999). The environmental fate of SM gaining importance with the passage of time due to its chronic heath toxic effects is associated with this agent. Hence, proper treatment plan including the application of drugs for the treatment of chronic effects is crucial (Karalliedde et al. 2000). It is now clear that apart from genetic mutations, there is the involvement of epigenetic factors such as DNA methylation and modification. So, gene therapy is valuable to treat the epigenetic alterations in the next generations due to SM (Korkmaz et al. 2008). As the cases of malignant tumors have been reported in the organs, especially in the respiratory and digestive organs, the application of chemotherapeutic agents will provide a significant decrease in the mortalities happening due to SM (Balali-Mood and Balali-Mood 2015). The use of antioxidant liposomes against the SM toxic effects on the skin of the exposed individuals will be beneficial due to the release of these formulations for a proper duration of time (Paromov et al. 2011). The application of food supplements is increasing; the correlation of anti-mutagenic effects of food additives and human cancer is interconnected. Therefore, the use of such foods originally derived from plant sources will be a good remedy in the treatment of cancer (Sayed et al. 2012).

Conclusion

SM is a chemical warfare agent, which had been used in wars for decades. Upon exposure, an individual exhibits

wide range of structural and functional defects in reproductive system such as imbalance and retarded secretion of reproductive hormones. Likewise, SM is responsible for the testicular damage and sexual organs malfunctioning, leading to severe male infertility. The chronic genotoxic and reproductive effects of SM have been reported even after 20 years in postwar troopers. The toxic effects of SM are genetically oriented and can transfer from parents to offspring. There are several mechanisms through which SM produces chronic genotoxic and reproductive effects. Our study has concluded that the chronic genotoxic and reproductive effects caused by SM are closely associated with DNA damage in first exposure. This DNA damage has an important role in chronic toxic effects in exposed patients. It is necessary to scientifically investigate the DNA damage due to SM in next generation. This will help in finding specific cellular response and to evaluate effective treatment strategies for the better treatment of genotoxic and reproductive effects caused by SM. Furthermore, in our study we have stated that the DNA damage and chronic genotoxic effects of SM are due to the formation of highly reactive species and OS; therefore, it is important to treat the SM-exposed patients with antioxidants in order to protect the functioning of the reproductive system from DNA damage. However, to design a comprehensive treatment plan for SM-exposed patients mostly depends on the severity of the disease, the prescribed dosage of used antioxidants and the delivery system of such antioxidants to the target organs. It is important to consider a much greater population to find a specific association between SM exposure and physical abnormalities among children in those areas, which have been exposed to the attacks of chemical warfare agents in the past. It is important to take in account the safety measures related to the long-term effects of drug treatment, because the prolong use of such drugs can adversely affect the DNA repair, mutations and also the epigenetic alterations.

Acknowledgements This article is the outcome of an in-house financially non-supported study.

Author contributions All authors have directly participated in the planning or drafting of the manuscript and read and approved the final version.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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