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DRDE-07: A POSSIBLE ANTIDOTE FOR SULPHUR MUSTARD TOXICITY

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Abstract – Chemical Warfare Agents are classified in various categories and vesicating agents are one among them. Vesicating agents are mostly mustard agents. Sulphur mustard which is chemically known as bis(2-chloro ethyl) sulphide (SM), was first used in World War-I and in recent past in Iran-Iraq war. Its possible use by the terrorist groups can't be overlooked in the present scenario. As the mode of its action is still lacking, no specific treatment is so far known against SM induced systemic toxicity. The major drawback with the development of antidote against sulphur mustard is low efficacy of the potential compounds *in vivo* models. This review summarizes the current update about the work done so far and the future stratagies.

Key words: Antidote, amifostine, cytotoxicity DRDE-07, flavonoids

INTRODUCTION

Sulphur mustard (SM) is chemically Bis-(2-chloroethyl) sulphide, a vesicating agent, was first used as a chemical weapon during the World War-I and in the recent past during the Iran -Iraq conflict (4). It is currently regarded as one of the biggest threats by terrorist (1,9,21,33,46). The production of SM does not require specialized technology and thus the danger of potential use by the terrorist group against the civilian population is considerable. Apart from the surprise attacks, there is also a risk of an accidental exposure to SM during the destruction of declared stockpile (47).

Despite extensive research work by the scientists we are still far from a specific antidote against sulphur mustard induced systemic toxicity. An effective prophylactic agent against SM is required especially for personnel engaged in the destruction of SM and during inspection by the Organisation for the Prohibition of Chemical Weapons (52). Research is also being carried out on the identification of better decontamination agents and antidotes for SM toxicity (Figure 1) (46,52). In this review we have focused on the current status and strategies being adopted for the development of an antidote and also future directions.

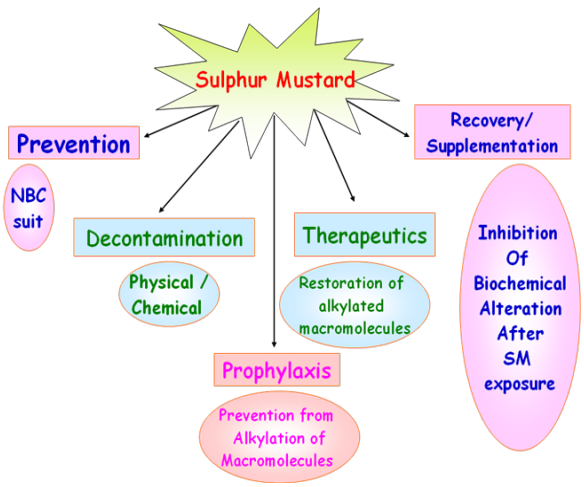


Figure 1. Schematic presentation of modes of protection against sulphur mustard toxicity.

SM TOXICITY AND POSSIBLE MECHANISM

All parts of the body that come in direct contact with the liquid or vapour are susceptible to the effect of SM. Eyes, skin and the respiratory tract are the principal targets of SM toxicity(33,34,50). The formation of thin-walled blisters is preceded by an asymptomatic latent period of several hours, followed by itching, pain

and erythema. Progression of the lesions and the degree of blistering and necrosis depend on delivered SM dose (10,37).

SM forms sulphonium ion in the body and alkylates DNA, leading to DNA strand breaks and cell death (2). Due to the high electrophilic property of the sulphonium ion, SM binds to a variety of cellular macromolecules (45). SM induces blisters at the site of exposure and is also a cytotoxic agent. These cytotoxic effects are manifested in widespread metabolic disturbances whose variable characteristics are observed in enzymatic deficiencies, vesicant action, abnormal mitotic activity and cell division, bone marrow depression and systemic poisoning (Figure 2) (9).

Alkylation of DNA is considered to be the most significant injury to cells from mustards. Oxidative stress is likely to be involved in the toxic effects following acute exposure (14,35). Alkylating agents are known to deplete glutathione (GSH) which contributes to lipid peroxidation and cell death (15). Apoptosis and necrosis has been reported as one of the consequences of SM injury in cell lines (8,20,36).

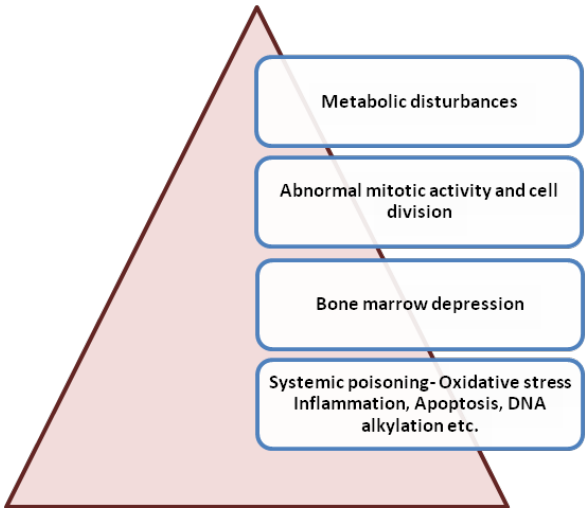


Figure 2. Schematic presentation of SM induced toxicity.

CURRENT STATUS OF TREATMENT FOR SM TOXICITY

Protease inhibitors and free radical scavengers have been previously suggested and tested as potential treatment for SM-induced injury with some success both *in vitro* and *in vivo* models (6,7,18,24). A large number of chemicals and drugs have been tested against sulphur and nitrogen mustard toxicity using *in vivo* and *in vitro* systems and some of them showed promising results (26,31,38). Several antidotes have been screened for reducing the systemic toxicity of SM in experimental animals (9,53,54). A variety of compounds tested to attenuate SM toxicity *in vitro* or *in vivo* include scavengers of SM and SM-induced oxygen radicals (15,24,30,53), inhibitors of cell death and promoters of cell survival (16,27,46,53) and numerous other pharmacological agents (26,39,40,41). Although benefits have been observed with some drugs in tissue culture systems, the antidotal activity of the test compounds was always too weak to be used as protectants against SM (30). The major problems associated with the antidote development against sulphur mustard toxicity include i) lack of information about its mechanism of action, ii) molecules reported to be protective *in vitro* system is always questionable *in vivo* model and iii) SM causes severe cytotoxicity with multi-organ failure.

STRATEGIES FOR THE DEVELOPMENT OF THE ANTIDOTE

Natural products

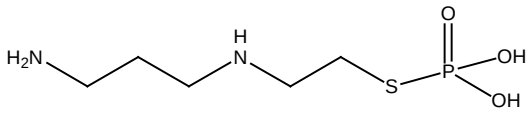
Flavonoids are polyphenolic compounds present in several plants, which inhibit lipid peroxidation and also act as a free radical scavenger (29). A number of flavonoids and herbal extracts were investigated but with limited success. Gossypin, quercetin, flavonoides extracted from *Hippophae rhamnoides* showed protection against percutaneouly administered SM (12,13). Whole plant extracts like alcoholic and water extracts of *Sea buckthorn* and *Aloe vera* gel also showed partial protection against SM toxicity in rodents (11,51). As discussed in these reports these flavonoids and herbal extracts were able to provide significant protection against biochemical alterations induced by mustard toxicity, its efficacy in terms of fold protection was always too weak.

Synthetic antidotes

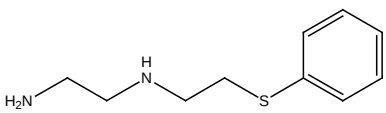
Among various scavengers, the radioprotectors play a promising role and some of them (WR-2721, WR-3689, and WR-638) were reported to have been tested against nitrogen mustard and sulphur mustard toxicity (17,33). Amifostine, earlier known as WR-2721 developed by Walter Reed Laboratory (USA), has been extensively used as a chemical radioprotector for the normal tissues in cancer

radiotherapy and chemotherapy (19,48). Amifostine is dephosphorylated to free thiol, which quickly enters the normal tissues and protects against alkylating agents and radiation (5). This protection has been attributed to the modulation of glutathione level and known to protect against radiation induced lipid peroxidation (49). SM is known as a radiomimetic agent therefore amifostine might be a promising antidote against SM also.

Amifostine and two of its homologues, which are essentially the S-substituted derivative of aminoalkylamino ethanethiols were synthesized and evaluated against SM toxicity. Initial screening of these compounds showed that amifostine is impressively active by i.p. and oral route, but the protection was less through oral route. These observations encouraged us to synthesise a series of S-substituted aminoalkylamino ethanethiols as potential antidotes against SM toxicity. In the same series we synthesized various other analogues and after evaluating their efficacy in animal model we concluded that DRDE-07 (S-2(2-aminoethylamino) ethyl phenyl sulphide) was found very effective as oral SM antidote (Figure 3). Amifostine and DRDE-07 protected only when they were administered as pretreatment *in vitro*. In vivo protection was also evaluated in mice with oral treatment of amifostine and DRDE-07 against percutaneously administered SM. The protection was dose dependent and effective only when the agents were administered either as a pretreatment or simultaneously with SM. Both *in vitro* and *in vivo* data suggest the promising role of DRDE-07 as prophylactic agents against SM poisoning (2,23,53).



Amifostine



DRDE-07

Figure 3. Structures of DRDE-07 and Amifostine.

The protection offered by amifostine and DRDE-07 was more pronounced in mice than rat. These molecules have better bioavailability in mouse than rat. Although the magnitude of protection was less in the rats, DRDE-07 offered better protection than that of amifostine (23). This may be due to the direct interaction of the metabolite of DRDE -07 formed in first pass metabolism with SM. The major routes of SM entry are the skin and respiratory tract. The primary target following inhalation exposure to SM vapor is the lung parenchyma (31). The active metabolite of amifostine and DRDE-07 are expected to be present in the lung parenchyma so as to neutralize SM vapor. It appears that sufficient concentration of the active metabolite is not reached and hence there was no protection, when SM was given by inhalation (28). Amifostine and DRDE-07 did not protect when SM was administered by subcutaneous route. When SM was administered through percutaneous route amifostine and DRDE-07 were effective and suggest that the mechanism of toxic effect of SM varies with different routes.

As DRDE 07 is an investigational drug and proposed to be introduced as a prophylactic agent, various pharmacological and toxicological safety studies are mandatory. The cardio-respiratory effect of DRDE-07 using variable doses were carried out; (i) to evaluate its safety profile and (ii) to ascertain whether the drug may cause a decrease in blood pressure leading to the reduction in SM absorption, when the latter was administered through the percutaneous route. No significant effect on the cardio-respiratory variables at low dose were observed but a sudden fall in the mean arterial blood pressure was expected at a high dose of DRDE-07 (25). A significant time dependent and dose dependent decrease in respiratory frequency was observed following oral administration of Amifostine but not with DRDE-07. A variety of drugs that act as the central nervous system depressant viz, general anesthetics, opioid analgesics, sedatives and hypnotics cause a depression of respiration and respiratory stimulants like doxapram cause an increase in the respiratory frequency and tidal volume. DRDE 07 does not have any depressant or stimulant action on the central nervous system (44).

Amifostine, DRDE 07, and their analogues were also screened against nitrogen mustard induced systemic toxicity. None of the compounds were found as promising antidote for nitrogen mustard toxicity. However, these compounds showed protection against the biochemical changes induced by nitrogen mustard than already recommended drugs like

amifostine, N-acetyl cysteine, sodium thiosulphate and melatonin. DRDE 30 and DRDE 35 gave better protection against HN-2 and NH-3 (42). These two compounds also have better safety in terms of LD_{50} by oral and intraperitoneal routes (32).

The probable role of DRDE-07 as a prophylactic agent against sulphur mustard toxicity may be due to its antioxidant, anti-inflammatory and cytoprotective property. DRDE-07 showed anti-inflammatory and cytoprotective property (3,42). It is expected that DRDE-07 may be metabolised in liver and its active metabolite has strong nucleophilic property to scavenge free radicals or electrophilic moieties (Figure 4). SM forms sulphonium ions inside the body and it is electrophilic nature.

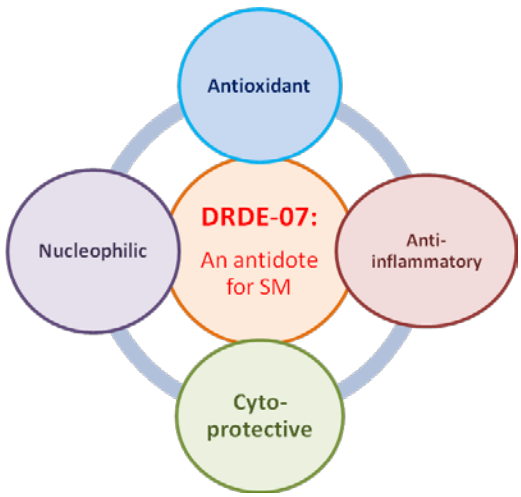


Figure 4. Possible mode of action of DRDE-07 against sulphur mustard toxicity.

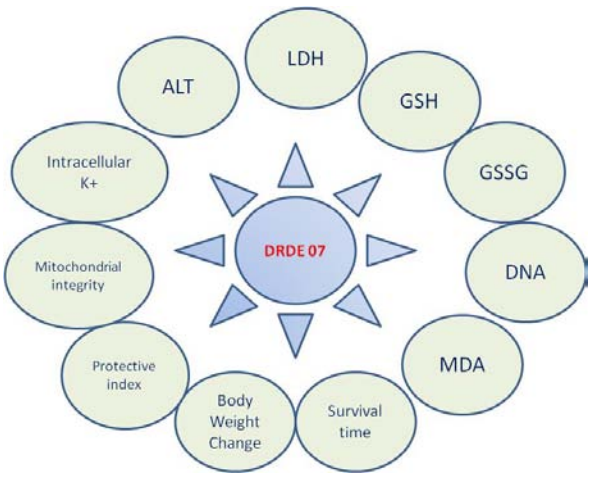


Figure 5. Biochemical and physiological alterations of sulphur mustard and protected by DRDE-07.

Future Strategies

(i) New DRDE-07 analogues- In search for more effective antidotes against SM, a series of novel S-2 (ω-aminoalkylamino) ethyl alkyl/aryl thioethers were synthesized which is water soluble for better oral efficacy and increase lipophilicity (Table 1). It has been observed that an increase in alkyl chain length increased the lipophilicity of the molecule and hence compound with the gradual increase in the carbon chain length at the sulphur atom were synthesized. For replacement of the phenyl moiety with cyclohexyl and substituted phenyl groups were selected. A number of compounds demonstrated significant protection against percutaneously administered SM (22). Few other analogues viz., DRDE-10, DRDE-21, DRDE-30 and DRDE-35 including DRDE-07 gave significant protection against systemic toxicity in mouse against percutaneous exposure (Fig 2). In the rat using same route, DRDE-07, DRDE-10 and DRDE-21 gave nearly two fold protection. Percutaneously administered SM significantly depleted the hepatic glutathione level, increased percent DNA fragmentation in mice. Few other analogues like DRDE-07, DRDE-30 and DRDE-35 significantly protected mice after SM intoxication. The histopathological lesions in liver and spleen induced by percutaneously administered SM were also reduced by pretreatment with these compounds. These classes of compounds though gave very good protection against SM but failed to give appreciable protection against 2-chloroethyl ethyl sulphide (CEES) and nitrogen mustard (NM), suggested that the mechanism and toxicity of the different mustard agents are different.

ii) Combination treatment – It is evident from above that DRDE-07, has promising role as a prophylactic agent against SM, despite the absence of phosphorothioate moiety like amifostine. In most of the studies the protection was seen only at a high dose of DRDE-07 and amifostine (25). Since the structures of amifostine and DRDE-07 are different and expected to act differently, we administered combination of DRDE-07 and amifostine in order to reduce the final dose. The other analogues DRDE-30 and DRDE-35 also showed good protection as a prophylactic agent against SM can also be combined with amifostine. This is a novel and interesting approach which requires further exploration using variable doses and/or route of administration.

Table 1. Structures of various analogues evaluated against SM toxicity (53).

Compound*	Structure
DRDE-07	NH_2-$(CH_2)_2$-NH-$(CH_2)_2$-S-C_6H_5
DRDE-09	NH_2-$(CH_2)_3$-NH-$(CH_2)_2$-S-C_6H_4-CH_3
DRDE-10	NH_2-$(CH_2)_2$-NH-$(CH_2)_2$-S-C_6H_4-CH_3
DRDE-21	NH_2-$(CH_2)_2$-NH-$(CH_2)_2$-S-C_6H_{11}
DRDE-30	NH_2-$(CH_2)_2$-NH-$(CH_2)_2$-S-$(CH_2)_2$-CH_3
DRDE-35	NH_2-$(CH_2)_2$-NH-$(CH_2)_2$-S-$(CH_2)_3$-CH_3

*All compounds are prepared as dihydrochlorides.

CONCLUSION

The combination therapy of drugs with structurally different molecules is a better approach in drug development because various pathways are involved. DRDE-07 is well investigated for SM treatment but has limitation because of its high dose. Amifostine also now a standard drug for radiation injury. Combination of DRDE-07 and amifostine may be a new treatment strategy against SM toxicity. Not only DRDE-07 few more analogues (DRDE-30 and DRDE-35) may also be promising at lower doses, with better safety profile, when administered in combinations. Further studies are required to get safer combination with low dose and more potent against SM toxicity.

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REFERENCES

1. Arroyo, C. M., Schafer, R.J., Kurt, E.M., Broomfield, C.A., and Carmichael, A.J., Response of normal human keratinocytes to sulfur mustard (HD), cytokine release using a non-enzymatic detachment procedure. *Hum. Exp. Toxicol.* 1999, **18:** 1-11.

2. Bhattacharya, R., Lakshmana Rao, P.V., Pant, S.C., Kumar, P., Tulsawani R.K., Pathak, U., Kulkarni, A. and Vijayaraghavan, R., Protective effects of amifostine and its analogues on sulfur mustard toxicity *in Vitro* and *in Vivo*. *Toxicol. Appl. Pharmacol.* 2001, **176:** 24-33.

3. Bhutia, Y.D., Vijayaraghavan, R., Pathak, U., Analgesic and anti-inflammatory activity of amifostine, DRDE-07 and their analogues in mice. *Ind. J. Pharmacol.* 2010, **42:** 17-20.

4. Black, R.M. and Pearson, G.S., Unequivocal evidence. *Chem. Br.* 1993, 299: 584-587.

5. Capizzi, R.L., The preclinical basis for broad spectrum selective cytoprotection of normal tissues from cytotoxic therapies by amifostine. *Semin. Oncol.* 1999, **26:** 3-21.

6. Cowan, F.M., Anderson, D.R., Broomfield, C.A., Byers, S.L. and Smith, W.J., Biochemical alterations in rat lung lavage fluid following acute sulfur mustard inhalation, II. Increase in proteolytic activity. *Inhal. Toxicol.* 1997, **9**: 53-61.

7. Cowan, F.M., Broomfield, C.A. and Smith, W.J., Suppression of sulfur mustard increased IL-8 in human keratinocyte cell cultures by serine protease inhibitors, implications for toxicity and medical countermeasures. *Cell Biol. Toxicol.* 2002, **18**: 175-180.

8. Dabrowska, M.I., Becks, L.L., Lelli, J.L., Levee, M.G., and Hinshaw, D.B., Sulfur mustard induces apoptosis and necrosis in endothelial cells. *Toxicol. Appl. Pharmacol.* 1996, **141:** 568-583.

9. Dacre, J.C. and Goldman, M., Toxicology and pharmacology of the chemical warfare agent sulfur mustard. *Pharmacol. Rev.* 1996, **48**: 290-326.

10. Evison, D., Hinsley, D. and Rice, P., Chemical weapons. *Brit. Med. J.* 2002, **324**: 332-335.

11. Gautam, A., Singh, S., Kulkarni, A.S., Pant, S.C. and Vijayaraghavan, R., Protective effects of *Aloe vera* L. Gel against sulphur mustard induced systemic toxicity and skin lesions. *Ind. J. Pharmacol.* 2005, **37:** 103-110.

12. Gautam, A. and Vijayaraghavan, R., Prophylactic effect of Gossypin against percutaneously administered sulphur mustards in mice. *Biomed. Environ. Sci.* 2007, **20:** 250-259.

13. Gautam, A., Vijayaraghavan, R., Pant, S.C., Om Kumar, Singh, S. and Satish Kumar, H.T., Protective effect of

quercetin against sulphur mustard-induced oxidative stress in mice. *Def. Sci. J.* 2007, **57:** 707-720.
14. Giuliani, I., Baeza-Squiban, A., and Marano, F., Early cytotoxic effects of chlorethamine, a nitrogen mustard, on mammalian airway epithelium. *Toxicol. In Vitro,* 1997, **11**: 695-702.
15. Gross, C.L., Innace, J.K., Hovatter, R.C., Meier, H.L., and Smith, W.J., Biochemical manipulation of intracellular glutathione levels influences cytotoxicity to isolated human lymphocytes by sulphur mustard. *Cell Biol. Toxicol.* 1993, **9**: 259-268.
16. Gross, C.L., Meier, H.L., Papirmeister, B., Brinkley, F.B. and Johnson, J.B., Sulphur mustard lowers nicotinamide adenine dinucleotide concentrations in human skin grafted to athymic nude mice. *Toxicol. Appl. Pharmacol.* 1985, **81:** 85-90.
17. Gray, P.J., A Literature Review on the Mechanism of Action of Sulphur and Nitrogen Mustard, *Defence Science and Technology Organisation (DSTO),* MRL-TR, 1989, **89:** 24.
18. Guignabert, C., Taysee, L., Calvet, J.H., Planus, E., Delamanche, S., Galiacy, S. and d'Ortho, M.P., Effect of doxycycline on sulfur mustard-induced respiratory lesions in guinea pigs. *Am. J. Physiol. Cell. Mol. Physiol.* 2005, **289:** L67-L74.
19. Hospers, G.A., Eisenhauer, E.A. and De Vries, E.G., The sulfhydryl containing compounds WR-2721 and glutathione as radio and chemoprotective agents. A review, indications for use and prospects. *Br. J. Cancer* 1999, **80:** 629-638.
20. Hur, G.H., Kim, Y.B., Choi, D.S., Kim, J.H. and Shin, S., Apoptosis as a mechanism of 2-chloroethylethyl sulphide-induced cytotoxicity. *Chem. Biol. Interact.* 1998, **110**: 57-70.
21. Kehe, K., and Szinicz, L., Medical aspects of sulphur mustard poisoning. *Toxicol.* 2005, **214**: 198-209.
22. Kulkarni, A.S., Vijayaraghavan, R., Anshoo, G., Satish, H.T., Pathak, U., Raza, S.K., Pant, S.C., Malhotra, R.C. and Prakash, A.O., Evaluation of analogues of DRDE-07 as prophylactic agents against the lethality and toxicity of sulfur mustard administered through percutaneous route. *J. Appl. Toxicol.* 2006, **26:** 115-125.
23. Kumar, P., Vijayaraghavan, R., Kulkarni, A.S., Pathak, U., Raza, S.K. and Jaiswal, D.K., In vivo protection by amifostine and DRDE-07 against sulphur mustard toxicity. *Hum. Exp. Toxicol.* 2002, **21:** 371-376.
24. Lindsay, C.D. and Hambrook, J.L., Protection of A549 cells against the toxic effects of sulphur mustard by hexamethylenetetramine. *Hum. Exp. Toxicol.* 1997, **16**: 106-114.
25. Malviya, V., Singh, R., Kumar, D., Pathak, U., Kumar, P., Jaiswal, D.K., Mathur, R., and Vijayaraghavan, R., Cardiorespiratory effects of DRDE 07, a new prophylactic agent for sulphur mustard toxicity, in anesthetized rats. *Ind. J. Pharmacol.* 2004, **36:** 234-237.
26. Mazumder, P.K., Sugendran, K. and Vijayaraghavan, R., Protective efficacy of calcium channel blockers in sulphur mustard poisoning. *Biomed. Environ. Sci.* 1998, **11:** 363-369.
27. Meier, H.L. and Johnson, J.B., The determination and prevention of cytotoxic effects induced in human lymphocytes by alkylating agent 2,2'- dichloroethyl sulfide (sulfur mustard, HD). *Toxicol. Appl. Pharmacol.* 1992, **113:** 234-239.
28. Nielsen, G.D., Mechanisms of activation of the sensory irritant receptor by airborne chemicals. *Crit. Rev. Toxicol.* 1991, **21:** 183.
29. Nijveldt, R.J., Nood, E., Hoorn, D.E.C., Boelen, P.G., Norren. K. and Leeuwen, P.A.M., Flavonoids: A review of probable mechanisms of action and potential applications. *Am. J. Clin. Nut.* 2001, **74:** 418-423.
30. Om Kumar, Sugendran, K. and Vijayaraghavan, R., Protective effect of various antioxidants on the toxicity of sulphur mustard administered to mice by inhalation or percutaneous route. *Chem. Biol. Interact.* 2001, **134:** 1-12.
31. Pant, S.C., Vijayaraghavan, R., Kannan, G.M., and Ganeshan, K., Sulphur mustard induced oxidative stree and its prevention by sodium 2,3-dimercaptopropane sulphonic acid (DMPS) in mice. *Biomed. Environ. Sci.* 2000, **13:** 225-232.
32. Pathak, U., Raza, S.K., Kulkarni, A.S, Vijayaraghavan, R., Kumar, P. and Jaiswal, D.K, Novel S-substituted aminoalkylamino ethanethiol a potential antidotes against sulphur mustard toxicity. *J. Med. Chem.* 2004, **47:** 3817-3822.
33. Papirmeister, B., Feister, A.J., Robinson, S.I. and Ford, R.D., *Medical defense against mustard Gas: Toxic mechanisms and pharmacological implications*, (CRC Press, Boca Raton, FL) 1991.
34. Pechura, C.M. and Rall, D.P., Veterans at risk: The health effects of mustard gas and lewisite (National Acadamy Press, Washington, D.C.), 1993, 428.
35. Rappeneau, S., Baeza-Suiban, A., Braut-Boucher, F., Aubery, M., Gendron, M.C., and Marano, F., Use of fluorescent probes to assess the early sulfhydryl depletion and oxidative stress induced by mechlorethamine on airway epithelium. *Toxicol. In Vitro.* 1996, **13**: 765-771.
36. Rosenthal, D.S., Simbulan-Rosenthal, C.M., Iyer, S., Spoonde, A., Smith, W., Ray, R., and Smulson, M.E., Sulfur mustard induces markers of terminal differentiation and apoptosis in keratinocytes via a Ca^{2+}-calmodulin and caspase-dependent pathway. *J. Invest. Dermatol.* 1998, **111**: 64-71.
37. Ruff, A.L., and Dillman, J.F., Signaling molecules in sulfur mustard-induced cutaneous injury. *Eplasty* 2007, 8.
38. Sawyer, T.W., Hancock, J.R., and D' Agostino, P.A., L-thiocitrulline: A potent protective agent against the toxicity of sulphur mustard in vitro. *Toxicol. Appl. Pharmacol.* 1998, **151:** 340-346.
39. Sawyer, T.W., Modulation of sulphur mustard toxicity by arginine analogues and related nitric oxide synthase inhibitors in vitro. *Toxicol. Sci.* 1998, **46:** 112-121.
40. Sawyer, T.W, Characterization of protective effects of L-nitroarginine methyl ester (L-NAME) against the toxicity of sulphur mustard in vitro. *Toxicol.* 1998, **131:** 21-32.
41. Sawyer, T.W. and Risk, D., Effect of selected arginine analogues on sulphur mustard toxicity in human and hairless guinea pigs skin keratinocytes. *Toxicol. Appl. Pharmacol.* 2000, **163:** 75-85.
42. Sharma, M., Vijayaraghavan, R. and Gautam, A., DRDE-07 and its analogues as promising cytoprotectants to nitrogen mustard (HN-2) - An alkylating anticancer and chemical warfare agent. *Toxicol. Lett.*, 2009, **188:** 243-250.
43. Sharma, M., Vijayaraghavan, R., Pathak, U. and Ganesan, K., Prophylactic efficacy of amifostine, DRDE 07and their analogues against percutaneously administered nitrogen mustards and sulphur mustard. *Def. Sci. J.* 2009, **59:** 512-516.
44. Singh, S., Vimal, M., Gautam, A., Singh, R., Pathak, U., Raza, S.K. and Vijayaraghavan, R., Respiratory effects of amifostine and DRDE 07, the probable prophylactic agents of sulphur mustard in rats. *Def. Sci. J.*, 2006, **56:** 531-541.
45. Somani, S.M. and Babu, S.R., Toxicodynamics of sulphur mustard. *Int. J. Clin. Pharmacol. Ther. Toxicol.* 1989, **27**: 419-435.

46. Sugendran, K., Kumar, P. and Vijayaraghavan, R., Treatment for sulphur mustard Poisoning- A Review. *Def. Sci. J.* 1998, **48:** 155-162.
47. Szinicz, L., Worek, F., Thiermann, H., Kehe, K., Eckert, S. and Eyer, P., Development of antidotes: problems and strategies. *Toxicol*. 2007, **233**: 23-30.
48. Uma Devi, P., Nagarathnam, A. and Satish Rao, B.S., In: *Introduction to Radiation Biology*, Chemical modifiers of radiosensitivity. 2000, pp. 131-150.
49. Uma Devi, P. and Prasanna, P.G.S, Radioprotective effect of combinations of WR-2721 and mercaptopropionylglycine on mouse bone marrow chromosomes. *Radiat. Res*. 1990, **124:** 165-170.
50. Vijayaraghavan, R., Modifications of breathing pattern induced by inhaled sulphur mustard in mice. *Arch. Toxicol*. 1997, **71:** 157-164.
51. Vijayaraghavan, R., Gautam, A., Kumar Om, Pant, S.C., Sharma, M., Singh, S., Satish, H.T., Singh, A., Nivsarkar, M., Kaushik, M.P., Sawhney, R.C., Chaurasia, O.P. and Prasad, G.B.K.S., The Protective effect of ethanolic and water extracts of *Hippophae rhamnoides* against the toxic effects of mustard gas. *Ind. J. Exp. Biol*. 2006, **44:** 821-826.
52. Vijayaraghavan, R, Kulkarni, A., Kumar, P., Lakshmana Rao, P.V., Pant, S.C., Pathak, U., Raza, S.K. and Jaiswal, D.K., In: *Pharmacological Perspectives of Toxic Chemicals and Antidotes* Prophylactic efficacy of amifostine and DRDE-07 against sulphur mustard administered through various routes. 2001, pp 25-39.
53. Vijayaraghavan, R., Sugendran, K., Pant, S.C., Husain, K. and Malhotra, R.C., Dermal intoxication of mice with bis(2-chloroethyl) sulphide and the protective effect of flavonoids. *Toxico*. 1991, **69:** 35-42.
54. Vojvodic, V., Milosavljevic, Z., Boskovic, B. and Bojanic, N., The protective effect of different drugs in rats poisoned by sulfur and nitrogen mustards. *Fundam. Appl. Toxicol*. 1985, **5:** S160-S168.