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SYNTHESIS AND CYTOPROTECTIVE EFFICACY EVALUATION OF NEW DRDE-07 ANALOGUES AGAINST SULPHUR MUSTARD TOXICITY

Shikha Sharma*[1], Neeti Jain [2], Uma Pathak [2], S C Pant [2] and R Vijayaraghavan [2]

Department of Pharmaceutical Sciences, Hari Singh Gaur University [1], Sagar (M.P.), India
Defence Research Development Establishment [2], Gwalior (M.P.), India

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Correspondence to Author:

Mrs Shikha Sharma

Department of Pharmaceutical Sciences, Hari Singh Gaur University, Sagar (M.P.), India

ABSTRACT

Sulfur mustard is well known as toxic Chemical warfare agent and on contact with skin it produces blisters as well as systemic toxicity. In this study we have evaluated the efficacy of few analogues of S-2(2-amino-ethylamino) ethyl phenyls sulphide (DRDE-07) and a known radioprotector amifostine against SM induced toxicity in mouse. We have selected seven newly synthesized analogues of DRDE-07 for their protection against $2LD_{50}$ dose of SM. On the basis of their protective efficiency we further evaluated the selected compounds using biochemical markers. Hepatic biochemical and histological assays were carried out in liver of SM exposed animals on day 3 and day 7 post treatment. Among the seven analogues DRDE-07, DRDE-19 and DRDE-46 showed a significant reduction in hepatic malondialdehyde levels compared to SM exposed liver, besides restoring the liver GSH level. Treatment with amifostine was also effective in regulating these biochemical parameters, The results thus conclude that oral administration of amifostine, DRDE-07, DRDE-19 and DRDE-46 were effective as prophylactic agent for protecting SM toxicity, and DRDE-07 proved to be comparatively better then others.

INTRODUCTION: Bis (2- chloroethyl) sulphide, commonly known as sulphur mustard or mustard gas (SM) is a frequently used chemical warfare agent and produces serious blisters on contact with human skin. It is an alkylating agent, mutagenic and a suspected carcinogen [1-3]. The eyes, skin and respiratory tract are the principle target organ of SM toxicity [4]. The main target of SM toxicity is skin due to sensitivity of frequently dividing basal cells. Thus the toxicity by a dermal route is more compared to oral or subcutaneous route [5, 6]. The blistering response on contact with SM may be due to release of proteas that damage glycoproteins such as lamilin which responsible for dermo-epidermal attachment [7]. In aqueous medium SM gets hydrolyzed to form cyclic ethylene episulfonium intermediate, which reacts with compounds containing nucleophilic functional groups viz., amino, sulfhydryl, carboxylic and hydroxyl, in proteins and nucleic acid.

These species give rise to reactive oxygen species (ROS) that further cause oxidative damage to a number of molecules in cell, including membrane lipids, proteins, and nucleic acids leading to cell death [8, 9]. The deleterious effects of ROS is measured by the amount of lipid peroxidation are controlled by cellular antioxidant defence system that include antioxidants such as reduced glutathione (GSH), enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) [10, 11] suggested that SM toxicity is initiated by depletion of GSH, a vital antioxidant that scavenges the highly reactive oxygen species. Acute exposure to SM may also induce severe local and systemic toxicity and incapacitation, thus it requires immediate specific antidotes other than the decontaminating agents [12]. Available literature suggests that we lack an effective antidote in acute SM poisoning. Many compounds have shown good prophylactic as well as therapeutic protection in-vitro but their in vivo efficacy is still questionable.[13-15] A variety of compounds tested in animal models to attenuate SM toxicity include scavenger of SM and SM-induced oxygen free radical inhibitors of cell death and promoter of cell survival or cytoprotectors along with various other pharmacological agents [16-19]. Among various SM scavengers, the radioprotectors play a promising role and amifostine is very well studied as cytoprotector in cancer radiotherapy and chemotherapy [20-23]. S- 2 (2- aminoethylamino) ethyl phenyl sulphide (DRDE-07) an analogue of amifostine (WR 2721) developed by Defence Research and Development Establishment is reported as a promising prophylactic agent against the SM toxicity and showed better protection compared to amifostine [24]. Sulfoxide may be one of the probable bio-transformed molecules of DRDE 07 *in-vivo.* To investigate this point we synthesized sulfoxides of the compounds that have been found to provide significant efficacy against SM. 2- pyridyl analogue of DRDE-07 was prepared with the aim of exploring the effect of heterocyclic nitrogen moiety. The effect of increasing hydrophobicity was studied by amyl and hexyl derivative.

In the present we screened seven newly synthesized DRDE analogues (**Table 1**) for their cytoprotective efficacy against the 2 LD_{50} (16.2 mg/kg) dose of SM applied dermally.

TABLE 1: DOSE OF VARIOUS COMPOUNDS FOR ANTIDOTES STUDIES

Compounds	Structures	*Dose mg\kg
DRDE-07	$NH_2CH_2CH_2NHCH_2CH_2$-S-C_6H_5	249
DRDE-19	$NH_2CH_2CH_2NHCH_2CH_2$-S-C_5H_4N	280
DRDE-40	$NH_2CH_2CH_2NHCH_2CH_2$-S-$CH_2CH_2CH(CH_3)_2$	243
DRDE-41	$NH_2CH_2CH_2CH_2NHCH_2CH_2SCH_2CH_2CH(CH_3)_2$	180
DRDE-42	$NH_2CH_2CH_2NHCH_2CH_2$-S-$(CH_2)_5CH_3$	255
DRDE-44	$NH_2CH_2CH_2NHCH_2CH_2$-S(O)$CH_2CH_2CH_3$	232
DRDE-45	$NH_2CH_2CH_2NHCH_2CH_2S(O)CH_2CH_2CH_2CH_3$	246
DRDE-46	$NH_2CH_2CH_2NHCH_2CH_2$-S(O)C_6H_5	263
Amifostine	$NH_2(CH2)_3NHCH_2CH_2$-SPO_3H_2	180

MATERIAL AND METHODS:

Chemicals: SM was synthesized in the Chemistry Division of the establishment and SM was found to be above 99% pure by gas chromatographic analysis. 5, 5'- Dithio- *bis*- (2- nitrobenzoic acid (DTNB), EDTA and thiobarbituric acid (TBA) were purchased from Sigma chemicals (USA). Other chemicals are of high purity and were purchased from Qualigens or E- Merck (India). Biochemical kits were of Ecoline procured from Merck (India). Reagents required for synthesis were obtained from commercial supplier (Aldrich/Fluka, USA)

Synthesis of compounds: The key intermediate aminoalkylamino ethyl bromides were prepared by the reported procedure (Piper et al 1969). DRDE-07 and amifostine were prepared by the reported method (Pathak et al 2004). For synthesis of N^1- [2- (Pyridin- 2- yl sulfanyl) - ethyl]- ethane- 1, 2- diamine. Trihydrochloride (DRDE-19), 25 mmol 2- Mercaptopyridine and aminoethylamino ethyl bromide dihydrobromide were mixed together and 75mol NaOH was added. Reaction mixture was heated at 90-95^oC for ten minute then compound was extracted with chloroform. After solvent removal, the residue was dissolved in ethanol and treated with concentrated HCl. The hydrochloride salt formed was further purified by recrystallisation from ethanol-acetone.

For synthesizing DRDE-40, DRDE-41, DRDE-42, a two necked round bottom flask equipped with $CaCl_2$ guard tube and magnetic stirring bar was charged with aminoalkylamino ethyl bromide (0.025 mol), alkyl mercaptan (0.30 mol) and sodium metal (2 gm) in benzene (50 ml). The temperature was maintained at 5^oC. Methanol (10 ml) was added drop wise with constant stirring for 1 hr. The contents were refrigerated for two hours. The white sodium bromide salt thus formed, settled down at bottom and was filtered off, yielded oil was purified by column chromatography. Finally, pure compound was obtained as white crystalline solid by Passing HCl gas to the solution of compound in acetone. To synthesis DRDE- 44, DRDE- 45, DRDE- 46, 0.025 mol of the sulfide (Pathak et al 2004) was taken in a round bottom flask with a condenser and dissolved in minimum amount of methanol. To this 0.05 mol of 30 wt % H_2O_2 was added and the reaction mixture was refluxed with stirring. After two hours additional 0.025 mol of H_2O_2 was added. Progress of the reaction was monitored by TLC (CH_3OH: $CHCl_3$: NH_4OH 3: 2: 1). On completion of the reaction solvent was removed under vacuum. Sulfoxide is obtained as white solid residue which is further purified by re crystallization from methanol-acetone.

NMR analysis: Chemical shifts are expressed as δ values (ppm) relative to TMS as internal standard for ^1H NMR. The NMR spectra were recorded on Bruker-Avance400MHz NMR Spectrophotometer. In most of the cases the compounds were identified by recording their pseudomolecular ion $(M+H)^+$ under Electro spray (ESI). TLC was performed using precoated aluminium sheets with silica gel $60F_{254}$ for purity check.

Pharmacological activity:

Animals: Randomly bred Swiss female mice (25-30g) from the Institute's animal facility were used for the study. The animals were housed in polypropylene cages under controlled experimental conditions with free assess to food (standard pellet diet, Ashirwad Ltd, India) and water until 2hr before and after experiment. The care and maintenance of the animals were as per approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India).This study has the approval of the Institutional Animal Ethical Committee.

Protective efficacy: Hair from the back of mice was closely clipped using a pair of scissors, 24 hr prior to the experimentation. In the first experiment the analogues were screened for the cytoprotective efficacy against 2 LD_{50} dose of SM (1LD_{50}= 8.1 mg/kg, percutaneously). Freshly prepared solution of amifostine, DRDE-07 and their analogues were prepared in double distilled water. The structure of the analogues and the dose used are given in table 1. The antidotes were administered using oral feeding cannula, 30 min before the dermal application of SM (2 LD_{50} dose in PEG-300). Each group consisted of 6 animals.

The diluted SM was smeared uniformly onto back side of the animals and animals were observed for 14 days for mortality and body weight changes. In the second experiment the analogues that showed better protection viz., DRDE-07, DRDE-19 and DRDE-46 were further evaluated for their protection on biochemical and histological studies. The antidotes were given orally 30 min prior to SM application (2 LD_{50}). Each group consisted of eight mice and four were sacrificed on 3rd day and four on 7th day post treatment. The animals were anesthetized with ether and blood was withdrawn from orbital plexus and then animals were sacrificed by cervical dislocation for various biochemical and histological evaluations.

Biochemical estimations: Blood biochemistry, hepatic glutathione and hepatic MDA were estimated after the exposure. Hepatic Glutathione concentration of tissue was assayed according to method [25]. In brief, 150 milligram of tissue was homogenized in 0.02 M EDTA buffer (pH 8.0) and 50% TCA was added to it. Supernatant was mixed with 0.4 M tris buffer (Ph 8.9), and 0.01 M DTNB (5, 5'- Dithio- *bis*- (2-nitrobenzoic acid) and absorbance was read at 410 nm. Hepatic lipid peroxidation was determined by measuring the level of thiobarbituric acid substance (TBARS) formed, using thiobarbituric acid (TBA), according to the method [26]. Blood Hb, RBC and WBC were analyzed by using Bechman Coulter Analyzer (USA). SGPT, SGOT, ALP and total protein concentrations were estimated by using kits (Ecoline, Merck).

Histological evaluations: A portion of the liver tissue sample was fixed in 10% neutral buffered formaline solution for 24 hr. After proper fixation, small pieces were processed by dehydration in graded series of alcohol, cleaned in xylene and embedded in paraffin wax. Multiple sections of 4-5µm thickness were prepared, and stained with hematoxylin and eosin for light microscopic examination. Lesions were marked and confirmed with that of control. The severity of the lesions was characterized by using Leica-orthoplan microscope and LEICA-Qwin-500 image analyzer.

Statistics: The data was analyzed by one-way ANOVA followed by student Newman Keuls multiple comparison test (Sigma Stat: SPSS, USA). A probability value of < 0.05 was considered statistically significant.

RESULTS

NMR: (DRDE 19): Yield 52%, ^{1}H NMR (CD_3OD, 400 MHz): δ 8.52 (m, 1H), 8.31 (m, 1H), 7.52 (d, 1 H), 7.45 (m, 1H), 3.60 (t, 2H), 3.41 (t, 2H), 3.35 (m, 2H), 3.28 (m, 2H); ESI: 198 $(M+H)^+$.

(DRDE 40). Yield 62%, ^{1}H NMR (CD_3OD, 400 MHz): δ 0.94 (d, 6H), 1.51-1.54 (m, 2H), 1.70-1-73 (m, 1H), 2.6 (t, 2H), 2.91 (t 2H), 3.31-3.36 (m, 2H), 3.39-3.45 (m, 4H). ESI: 191 $(M+H)^+$.

(DRDE 41)63%, ^{1}H NMR (CD_3OD, 400 MHz): δ 0.93 (d, 6H), 1.46-1.53 (m, 2H), 1.69 (m, 1H), 2.10-2.15

(m, 2H), 2.63 (t, 2H), 2.87 (t, 2H), 3.08 (t, 2H), 3.17 (t, 2H), 2.25 (t, 2H). ESI: 205 $(M+H)^+$.

(DRDE 42): 64%, ^{1}H NMR (CD_3OD, 400 MHz): δ 0.86 (t, 3H), 1.29-1.35 (m, 4H), 1.40-1.48 (m, 2H), 1.58-1.64 (m, 2 H), 2.63 (t, 2 H), 2.90 (t, 2H), 3.30-3.39 (t, 2H), 3.41-3.44 (m, 4 H). ESI: 205 $(M+H)^+$.

(DRDE 44): Yield 74%, ^{1}H NMR (CD_3OD, 400 MHz): δ 1.24 (t, 3H), 1.86-1.90 (m, 2H), 3.22-3.47 (m, 10H). ESI: 179 $(M+H)^+$.

(DRDE 45): Yield 77%, ^{1}H NMR (CD_3OD, 400 MHz): δ 1. 03 (t, 3H), 1.48-1.59 (m, 2H), 1.76-1.87 (m, 2H), 2.9-3.32 (m, 6H), 3.37-3.40 (m, 2H), 3.45-3.47 (m, 2H). ESI: 193 $(M+H)^+$.

(DRDE 46): Yield 78%, ^{1}H NMR (CD_3OD, 400 MHz): δ 3.42-3.60 (m, 8H), 7.66-7.70 (m, 3H), 7.80-7.82 (m, 2H); ESI: 213 $(M+H)^+$.

Pharmacological activity: In the initial screening for prophylactic efficacy (30 min pretreatment) of DRDE-07 analogues, revealed that DRDE-07, DRDE-19, and DRDE- 46 gave considerable protection against 2 LD_{50} doses of SM as evidenced by delayed mortality in these groups (**Table 2**).

TABLE 2: ANIMAL SURVIVAL UP TO 14 DAYS 2 LD_{50} SM APPLICATION ALONG WITH SYNTHESIZED COMPOUNDS

Agent	Days after SM administration (number of mice survived (cumulative) out of six)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	6	6	6	6	6	6	6	6	6	6	6	6	5	5
SM	6	6	6	6	5	0	0	0	0	0	0	0	0	0
DRDE-07+ SM	6	6	6	6	6	6	5	5	5	5	5	5	5	5
DRDE-19+ SM	6	6	6	6	6	6	5	5	5	5	5	5	4	4
DRDE-40+ SM	6	6	5	5	3	2	1	0	0	0	0	0	0	0
DRDE-41+ SM	6	6	6	4	4	4	3	2	0	0	0	0	0	0
DRDE-42+ SM	6	6	5	4	4	2	1	0	0	0	0	0	0	0
DRDE-44+ SM	6	6	6	6	4	4	4	3	3	3	1	1	0	0
DRDE-45+ SM	6	6	6	4	4	4	3	2	1	1	0	0	0	0
DRDE 46+ SM	6	6	6	6	6	6	6	5	4	4	4	4	4	4
Amifostine + SM	6	6	6	6	6	4	4	2	2	0	0	0	0	0

The body weight of mice administered with 2 LD_{50} dose of SM (16.2 mg/kg) decreased significantly compared to the control group. The body weight started decreasing after 24 h post exposure and decrease was significant on 3rd day post exposure. Due to sever loss of body weight the mice appeared emaciated. Among all the seven analogues, DRDE- 07, DRDE-19 and DRDE-46 protected the decrease in body weight significantly (**Fig. 1**). SM administration showed an increase in RBC count and Hb content. Partial protection was observed with DRDE-07, DRDE-19 and DRDE-46 with invert RBC count. The increase in Hb content was not altered in SM exposed animals pretreated with various antidotes. SM also showed an increase in SGPT, SGOT and ALP levels and the analogs partially protected there liver toxicity enzyme markers (**Table 3**).

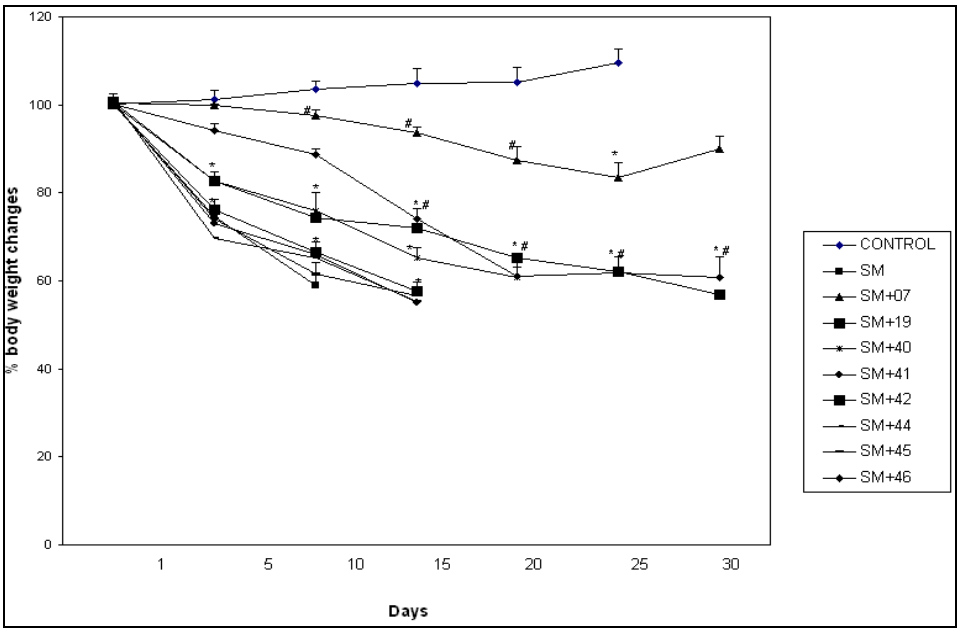


FIG. 1: EFFECT OF VARIOUS TREATMENTS ON % BODY WEIGHT IN SM EXPOSED IN MICE

TABLE 3: EFFECT OF 2 LD $_{50}$ OF PERCUTANEOUSLY ADMINISTERED SM ON SGPA, SGOT, ALP, RBC, Hb AND PROTECTION BY DRDE SERIES OF COMPOUND

Treatments	SGPT (IU/L)		SGOT (IU/L)		ALP (IU/L)		% RBC		% Hb	
	3rd day	7th day	3rd day	7th day	3rd day	7th day	3rd day	7th day	3rd day	7th day
Control	104.6±0.9	100.2± 1.9	100.1±1.2	102.1±0.5	103.1±2.9	100.9± 1.9	100.9 ± 3.2	102.9 ± 3.9	100.9 ± 1.5	103.6 ± 2.3
SM	140.6±1.3[a]	144.2± 1.4[a]	157.4±4.9[a]	184.3±9.5[a]	161.2±3.7[a]	174.4± 5.3[a]	135.8 ± 2.6[a]	151.3 ± 2.3[a]	118.7 ± 3.7[a]	126.1 ± 3.8[a]
SM+DRDE-07	108.8±4.9[b]	100.3±3.5[b]	130.2± 2.3[b]	108.2±2.8[b]	110.3±5.2[b]	100.6±2.8[b]	113.7 ± 5.3	107.2 ± 4.7	106.9 ± 7.8	103.4 ± 5.5
SM+DRDE-19	112.3±1.1[b]	108.6±5.9[b]	128.3± 3.6[b]	113.3±3.9[b]	118.8±3.9[b]	108.3±4.4[b]	118.4 ± 4.6[a]	106.3 ± 7.3	108.7 ± 4.5	105.4 ± 2.9
SM+DRDE-46	118.6±1.5[b]	115.4±3.5[b]	138.1±4.1[b]	117.4±4.1[b]	119.6±4.1[b]	110.5±3.6[b]	119.8 ± 3.7[a]	108.6 ± 9.3	112.7 ± 2.7	110.2 ± 2.8
Amifostine	116.9±1.1[b]	112.3±5.2[b]	134.3±3.9[b]	111.3±2.9[b]	116.8±2.9[b]	111.7±5.1[b]	126.4 ± 4.8[a]	104.3 ± 4.8	112.7 ± 3.9	107.5 ± 2.1

Control values: RBC=8.4× 10^6 cells/µl; Hb=13.2 ± 0.4 g/dl; SGPT- 25±1.0 IU/L, SGOT - 26±1 IU/L, ALP - 29±2.1IU/L; Significance P<0.05; a-Control versus treatment; b-SM versus treatment

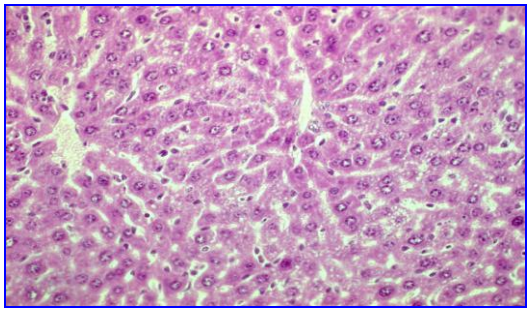
Table 4 shows the effect of various treatments on hepatic TBAS and GSH level. The TBAR level was increased significantly in SM treated animals. Treatment with the analogues also showed significant increase in MDA level compared to control animals on day 3whereas on day 7 a significant reduction in liver MDA was observed. SM exposed animals showed a significant reduction in reduced glutathione level in liver tissue on 3rd day while pretreatment with DRDE-07, DRDE-19, DRDE- 46 and amifostine showed a significant increase in liver GSH level. In Histological evaluation, liver section of control mice showed normal cord pattern, hepatic lobule, central canal and hepatocytes (**fig. 2-a**).

SM administered animals on day 3 showed severe vacuolar degeneration of hepatocytes with perinuclear clumping of cytoplasm and condensation of chromatin material indicative of cell death (**fig 2-b**). Pretreatment with DRDE-07 significantly blocked the hepatic degeneration. Mice liver, pretreated with DRDE-19 showed pyknosis and sporadic necrosis in hepatic parenchyma. In the case of DRDE-46 regressed glanulovacuolor degeneration and perinuclear clumping of cytoplasm was observed. SM administrated mice liver pretreated with amifostine showed minimal to moderate lesions and some hepatocytes were showed granular degeneration (**Figure 2 c-e**). On 7th day post SM administration, liver histology of mice showed sever centric lobular degeneration of hepatocytes along with congestion and hyper activation of kupffer cells (**Fig. 3-b**). Sever degenerative changes were not observed in DRDE-07 treated mice (**fig. 3-c**). Moderate to sever lepidoses and accumulation of fibriroid material were observed in animals treated with DRDE-19 (**fig. 3-d**). Pretreatment with DRDE-46 further blocked the progression of lesions caused by SM application on 7th day post treatment (**Fig. 3-e**). Amifostine treatment also blocked the lesion caused by SM as changes were minimal to mild (**fig. 3-f**).

TABLE 4: EFFECT OF 2 LD $_{50}$ OF PERCUTANEOUSLY ADMINISTERED SM ON GSH, MDA AND PROTECTION BY DRDE SERIES OF COMPOUND

Treatments	% MDA		% GSH	
	3rd	7th	3rd	7th
Control	100.2±2.1	100.4±1.3	100.6±2.9	100.9±1.8
SM	145.2 ±3.9 [a]	165.7 ±5.2 [a]	54.6 ±2.1 [a]	31.9 ±1.7 [a]
DRDE-07+SM	125.1 ±1.1 [a]	108.8 ±2.9 [b]	67.9 ±7.9 [b]	97.1 ±5.9 [b]
DRDE-19+SM	131.4 ±4.5 [a]	110.9 ±5.2 [b]	71.6 ±1.1 [b]	88.3 ±6.2 [b]
DRDE-46+SM	141.3 ±1.9 [a]	113.6 ±3.9 [b]	64.8 ±2.6 [b]	84.9 ±2.9 [b]
Amifostine+SM	139.8 ±3.8 [a]	123.6 ±4.1 [b]	66.8 ±0.7 [b]	88.9 ±2.6 [b]

Control values: MDA= 3.86 ± 0.10 n moles/g tissue; GSH =3.50 ± 0.1 µ moles/g tissue; Significance P<0.05; a-Control versus treatment; b-SM versus treatment



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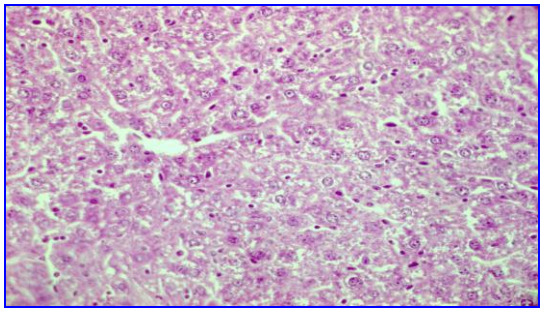

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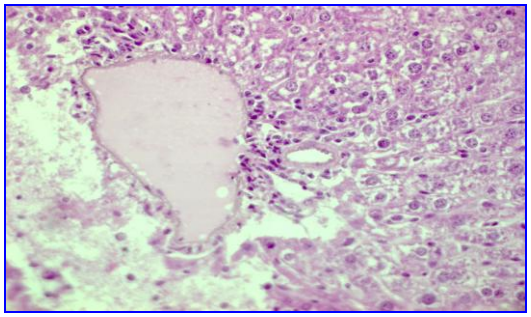

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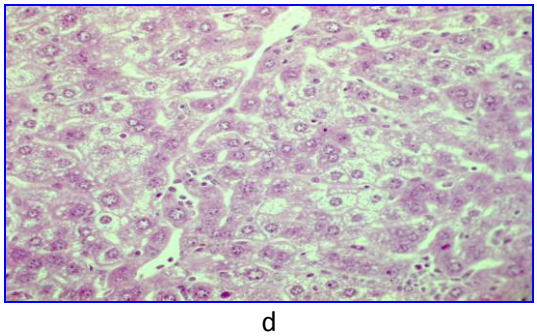

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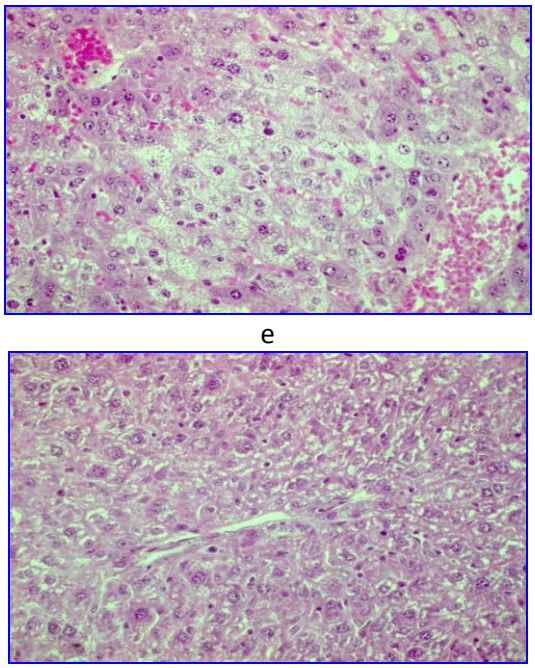

FIG. 2: PHOTOMICROGRAPHS OF CONTROL AND SULFUR MUSTARD ADMINISTERED (16.2 MG/KG, PC) MICE LIVER AND ITS PROTECTION BY VARIOUS COMPOUNDS, DAY 7TH DAY AFTER ADMINISTRATION, H X E, 100X

a) Control b) Control+ SM c) DRDE-07+ SM d) DRDE-17+SM e) DRDE-46+SM f) Amifostine +SM

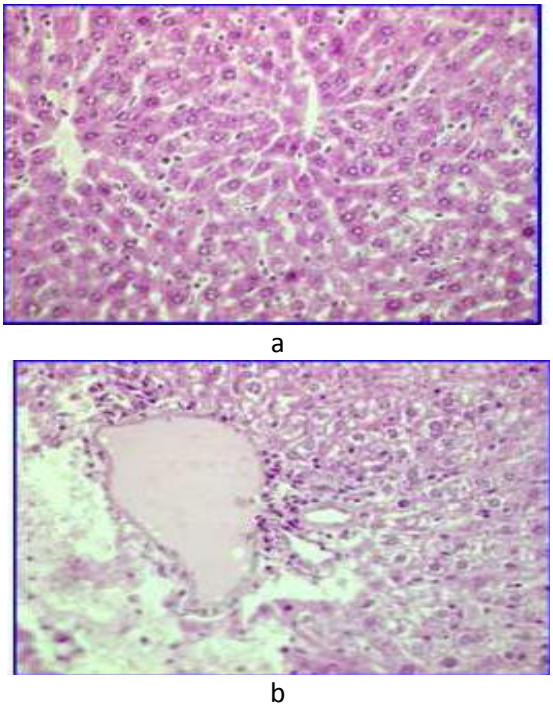

FIG. 3: PHOTOMICROGRAPHS OF CONTROL AND SULFUR MUSTARD ADMINISTERED (16.2 MG/KG, PC) MICE LIVER AND ITS PROTECTION BY VARIOUS COMPOUNDS, DAY 7TH DAY AFTER ADMINISTRATION, H X E, 100X

a) Control b) Control+ SM c) DRDE-07+ SM d) DRDE-17+SM e) DRDE-46+SM f) Amifostine +SM

DISCUSSION: SM is a lipophilic compound and is more toxic by the dermal route compared to oral and subcutaneous route due to fast absorption at skin site and formation of more active metabolite [27]. In this study we have evaluated seven newly synthesized analogues DRDE- 07 of along with amifostine for their protective efficiency and studied the effect of these antidotes on various biochemical markers that plays the crucial role in SM toxicity. The survival of animals after SM exposure was observed for 14 days, as beyond this period animals are reported to gain weight [12].

Survival pattern and body weight alteration showed that among all the screened antidotes only three (DRDE-07, DRDE-19, DRDE-46) are offered protection against the SM toxicity at 2 LD_{50} dose. Amifostine is a known radioprotector and chemoprotector agent [4, 20]. It provides protection in dose dependent manner and expected to neutralize and reduce the concentration of SM present inside the cells [23]. It is postulated that free thiol formed due to dephosphorylation of amifostime scavenges the sulphonium ions, generated by the hydrolysis of SM and render it to less toxic compounds. It is reported that among the previously synthesized amifostine analogues, DRDE-07 showed more protection at lower doses compared to amifostine [23].

The present study also confirm that various alterations done at DRDE-07 molecule, the protection is not increased except in the case of DRDE-30 and DRDE-35 where alkyl substitution has been done, which may increase the absorbance of drug by cells [24]. SM is well known DNA alkylating agent, but the toxicity of SM is not only due to its alkylating properties [28]. Apart from DNA alkylation SM toxicity is also due to depletion of glutathione that acts as an alternative intracellular site or 'scavenger' for SM [17, 27]. GSH scavenges oxidative agents and its depletion is related to cytotoxicity. Reduction in GSH gives rise to free radical generation that reacts with membrane phospholipids and initiate lipid peroxidation and formation of MDA. In present study also the liver of SM exposed mice showed a significant reduction in GSH level and an increase in MDA level indicating the damage caused due to free radicals generated by SM toxicity. DRDE-07, DRDE-19 and DRDE-46 increased the level of GSH on day 3 and day7, and this GSH could be responsible for the reduction in MDA level on day7 after SM exposure. SM readily gets absorbed from site of contact and enters into circulation and produces systemic toxicity. Liver is one of the major known target organs to percutaneously administered SM, as liver is having rapidly dividing hepatocytes. Serum enzymes and total protein concentration are likely to change in various clinical diseases. They are treated as very good tools for diagnosis, prognosis and evaluation of treatment therapy.

Serum alkaline phosphatase (ALP), serum gultamic pyruvic transferase (SGPT) and serum glutamic oxaloacetic transferase (SGOT) are enzymes commonly used for liver cell integrity [29]; SM exhibit hepatotoxicity indicated by leakage of enzymes like SGPT, SGOT, ALP and histological observations [30]; in this study, 30 min prior oral administration of DRDE-07, DRDE-19 and DRDE-46 potentially protects all SGPT, SGOT and ALP leakage from liver tissue. DRDE-07 and DRDE-19 were found to be effective than amifostine. As liver is the main organ for detoxification and the protection offered by analogues may be related to their hepatoprotective activity, since all these analogues are developed from amifostine which is reported to posses a very good hepatoprotective activity. It is reported that SM acts via inflammatory pathway 31. Liver tissue of SM exposed animal showed strippling of

basophils and dissolution of nucleus with chromatin material in various hepatocytes. DRDE analogues minimized the degranulation of hepatocytes, basophilic stripling and perinuclear clumping of cytoplasm. The anti-inflammatory property of these analogues could be responsible for the protective properties. Prophylactic effect of DRDE-07 is better than amifostine against SM toxicity. This could be due to presence of aryl group in DRDE-07 and heterocyclic ring in DRDE-19. These compounds are having good lipophilicity that results in better bioavailability at the site of action [23]. Earlier it was reported substitution of propyl (DRDE-30) and butyl (DRDE-30) in the place of aryl also was found to be effective [24].

Our previous reports showed that amifostine and DRDE-07 provide protection against topically applied SM as well as *in-vitro* system also [22]; screening these antidotes against nitrogen mustard also will be required, so that they can be used to reduce the cytotoxicity of the anticancer mustard agents.

CONCLUSION: The present data indicate that DRDE-07 provides better cytoprotective activity against SM toxicity compared to other analogues. Biodistribution and pharmacokinetics studies may give more information to modify the compounds that can give more protection. The order of protection offered by various compounds are DRDE07> DRDE19> DRDE46> amifostine.

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