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Effect of Topically Applied Sulphur Mustard on Haematological, Biochemical & Histological Parameters in Mice

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ABSTRACT

Sulphur mustard (SM), a potent blistering agent, has been frequently used as a chemical weapon. In the present study, the effect of topically applied SM on haematological, biochemical and histological parameters in mice were investigated over a period of seven days. The application of SM resulted in significant increase of blood haemoglobin concentration, packed cell volume and erythrocyte count as well as plasma urea, uric acid and cholesterol levels. There was a significant decrease in blood leukocyte count, plasma total protein and albumin. There was significant increase in bleeding, clotting and prothrombin time. Elevated levels of alkaline phosphatase, lactate dehydrogenase, aspartate and alanine aminotransaminases, and creatine phosphokinase activities were also observed, revealing SM-induced liver damage and general state of illness. Histopathological observations revealed a mild to moderate degree of congestion and haemorrhage in the viscera examined. Also, there were mild degeneration and obliteration of chromatin material in liver and kidneys.

1. INTRODUCTION

Bis-(2-chloroethyl) sulphide, commonly known as sulphur mustard (SM) or mustard gas, is a potent blistering agent and frequently used chemical warfare agent[1]. It was the major cause of casualities resulting from the use of chemicals in World War I. Its use in regional conflicts over the past 50 years, most recently in the Iran/Iraq conflict in the Middle East has been documented. Though the Chemical Weapon Convention is going to be effective in due course of time, the possibility of its clandestine use always exists, necessitating the studies on the mechanism of its toxicity to achieve suitable medical protection measures.

It is an alkylating agent and is antimitotic, mutagenic, carcinogenic and cytotoxic. SM reacts in an aqueous phase with compounds containing nucleophilic functional groups, such as sulfhydryl, carboxyl, amino and hydroxyl groups of proteins, nucleic acids, etc. and its toxicity is considered to be due to its reactivity with one or more of the cell constituents[1].

Eventhough SM was found to reach systemic circulation and various organs in a human being after exposure[2], the systemic toxicity studies of SM are very much lacking. Recently, the authors have reported topically applied SM-induced alterations in systemic carbohydrate metabolism[3] and lipid peroxidation in the liver of mice[4]. In the present study, the influence of a single dose of topically applied SM on various haematological, biochemical and histological parameters over a period of seven days was carried out in mice.

2. MATERIALS & METHODS

Sulphur mustard (98 per cent purity) was synthesised in the Chemistry Division of the Defence Research & Development Establishment and characterised by proton NMR, IR and mass spectrometry. The SM was dissolved in polyethylene glycol 300 (PEG 300) for topical application. The percutaneous LD_{50} of SM in mice was 154.7 mg/kg[3]. All other chemicals used were of analytical grade (BDH or E. Merck or Sigma).

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Sixty male Swiss albino mice, bred in this Laboratory weighing between 20 and 30 g and maintained on a standard diet, were used for the experiments. All the animals were put on fast overnight prior to experimentation, while water intake was allowed *ad libitum*. A group of twelve mice was used as the control group; a group of eight mice was used for the haematological and biochemical studies and that of four mice was used for the histopathological studies. The remaining forty-eight mice were randomly divided into six groups of eight each for each duration of the study employing two doses of SM. The SM diluted in PEG 300 was applied percutaneously at a dose of 0.5 LD_{50} and 1.0 LD_{50} on the back side of mice after closely clipping the hair[3]. In the control group, the vehicle PEG 300 was applied percutaneously. Blood was drawn in heparinised tubes through retro-orbital plexus for various haematological and biochemical variable evaluations on days 1, 3 and 7 after SM application.

Blood haemoglobin (Hb) concentration was determined by the standard cyanmethaemoglobin method and packed cell volume (PCV) by micro-haematocrit method. Total leukocytes were counted by light microscopic examination using Neubauer chamber[5]. The bleeding time, (bleeding due to a glass capillary puncture of the orbital plexus), clotting time, prothrombin time and platelet count were determined by standard methods as described by Dacie and Lewis[6]. Standard colorimetric methods were used for the determination of plasma albumin[7], total protein[8], urea[9], uric acid[10] and cholesterol[11]. Creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate amino-transaminase (AST), and alanine aminotransaminase (ALT) were determined in plasma using Ranbaxy diagnostic kits[12].

For the histopathological studies, mice (four from each group of control, 0.5 LD_{50} and 1.0 LD_{50} were sacrificed by cervical dislocation on the third day after SM application or the vehicle in the control group. They were subjected to initial postmortem and later histo-pathological examination. The lung, liver and kidneys were removed at autopsy, fixed in 10 per cent neutral buffered formalin, embedded in paraffin, sectioned at 5-7 m thickness and stained with haematoxylin and eosin and also toluidine blue. Statistical analysis on the data was done by Student's 't' test and the level of significance was set at $P<0.05$.

3. RESULTS

Among the treated mice, three died within five to seven days after SM application in 1.0 LD_{50} group. No deaths occurred in both the control and the 0.5 LD_{50} groups. While the mean blood Hb concentration, PCV and RBC count increased significantly in 1.0 LD_{50} SM-treated group (Table 1), there was a significant gradual decrease in WBC count as the period after SM treatment advanced from day zero to seventh day.

Table 1. Effect of SM on blood haemoglobin (Hb), packed cell volume (PCV), erythrocytes (RBC) and leukocytes (WBC) in mice.

Group	Period after SM	Hb (g %)	PCV (vol %)	RBC ($x10^6/mm^3$)	WBC ($x10^3/mm^3$)
Control		13.9±0.19	41.7±0.33	4.3±0.12	7.7±0.08
0.5 LD_{50} SM	1 day	13.9±0.66	41.2±0.62	4.6±0.63	7.5±0.19
	3 days	14.9±0.27	42.8±0.09	4.8±+.07*	6.8±0.18*a
	7 days	14.8±0.41	43.6±0.26	4.8±0.13*	6.1±0.16*a
1.0 LD_{50} SM	1 day	14.9±0.16*	45.6±0.12*	4.6±0.07*	7.1±0.06*
	3 days	16.7±0.52*	49.3±0.86*	4.9±0.16*	6.7±0.05*a
	7days$	16.9±0.48*	50.2±0.58*	4.9±0.09*	5.4±0.05*a

Values are mean ±SE, n = 8;
$ Average of five readings (survived mice);
* Statistically significant as compared to the control group; and
a Statistically significant as compared to previous time interval [P<0.05]

Topical application of SM led to significant alterations in blood coagulation parameters without any appreciable change in platelet count (Table 2).

Table 2. Effect of SM on blood coagulation parameters

Group	Period after SM	Bleeding (min)	Clotting time (min)	Prothrombin time (s)	Platelets ($x\ 10^5/cm^3$)
Control	-	5.2±0.27	7.6±0.25	14.5±0.33	1.5±0.28
0.5 LD_{50} SM	1 day	5.5±0.26*	7.9 ±0.29	14.9±0.32	1.6±0.26
	3 days	6.4±0.33*	8.8±0.31*	15±0.29*	1.5±0.32
	7 days	6.4±0.38*	9.2±0.30	15.9±0.35*	1.4±0.32
1.0 LD_{50} SM	1 day	6.6±0.29*	8.6±0.22*	15.9±0.34*	1.4±0.35
	3 days	6.9±0.31*	9.1±0.32*	16.2±0.25*	1.5±0.33
	7 days$	7.2±0.30*	9.7±0.26*	17.5±0.31*	a1.6 ±0.31

Values are mean ± SE, n = 8;
$ Average of five readings (survived mice);
* Statistically significant as compared to control; and
a Statistically significant as compared to previous time interval [P<0.05]

There were significant increase in bleeding time, clotting time and prothrombin time progressively from first to seventh day after SM intoxication.

Total plasma protein and albumin also decreased significantly on third and seventh day following SM application (Table 3). Topically applied SM-induced statistically significant increase in the plasma levels of urea on the third and seventh days, and of uric acid and cholesterol at all the three time intervals (Table 3).

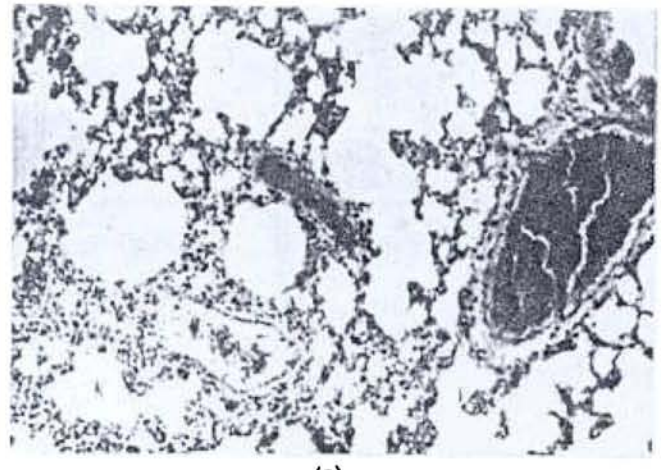
There was significant increase in plasma ALP and CPK levels at all the three periods but the increase in LDH and GOT activities after first and third day of SM application was not significant.

Table 3. Effect of SM (1.0 LD_{50}) on various biochemical parameters in mice.

Parameter	Control	SM treated Day 1	3	7[$]
Albumin (g%)	3.9±0.21	3.6±0.13	3.4±0.06*	3.3±+0.07*
Total protein (g%)	7.0±0.34	6.8±0.22	5.1±0.18*	4.±7+0.15
Urea (mg %)	32.5±1.16	33.5±2.17	38.6±2.21*	57.0±2.99*a
Uric acid (mg %)	0.9±0.06	1.7±0.32[1]	2.0±0.39*	1.5±0.29
Cholesterol (mg %)	77.8±1.80	109.9±2.40*	136.8±5.36*a	192.2±4.70*a
LDH (IU/L)	386±35.9	546±90.7	560±95.6	337±11.3
ALP (IU/L)	10.5±0.48	19.2±1.40*	44.7±1.81*a	48.7±2.62*
CPK (IU/L)	98±17.5	236±28.5*	273±42.8*a	37.3±39.3*
AST (IU/L)	25.5±1.22	25.2±2.30	29.3±3.51	27.2±1.46
ALT (IU/L)	24.8±1.82	25.6±2.40	28.7±3.2	26.6±2.46

Values are mean ± SE, n=8;
[$] Average of five readings (survived mice);
* Statistically significant as compared to the control group; and
[a] Statistically significant as compared to previous time interval [P<0.05]

Histopathological changes observed in the viscera of SM-intoxicated mice were qualitatively similar in both the groups except for the variation in the intensity of the effects, being lesser in the 0.5 LD_{50} group. The lung of SM-intoxicated mice showed a mild to moderate congestion and patchy haemorrhage (Fig. 1). The salient lesions in liver were mild granulovacuolar degeneration of hepatocytes with perinuclear clumping of cytoplasm and obliteration of chromatin material (Fig. 2). There were also a moderate number of individual necrotic cells in the centrilobular area. The histological lesions in kidneys of SM-intoxicated mice were congestion, haemorrhage and moderate degeneration, and sloughing off epithelium of convoluted tubules in renal cortex (Fig. 3). Obliteration of chromatin material was also observed in the kidneys.



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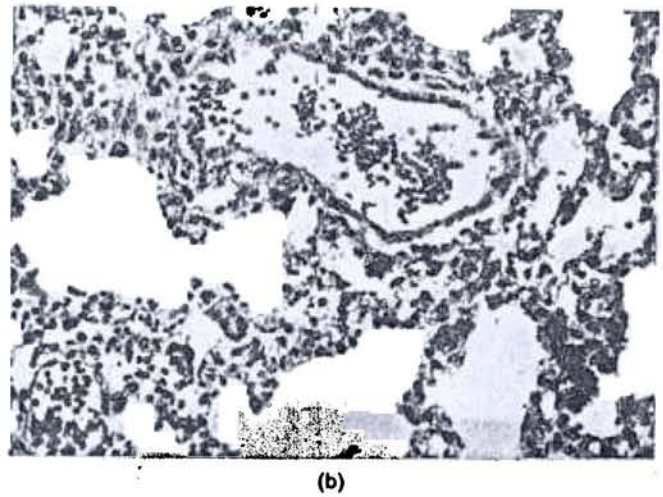
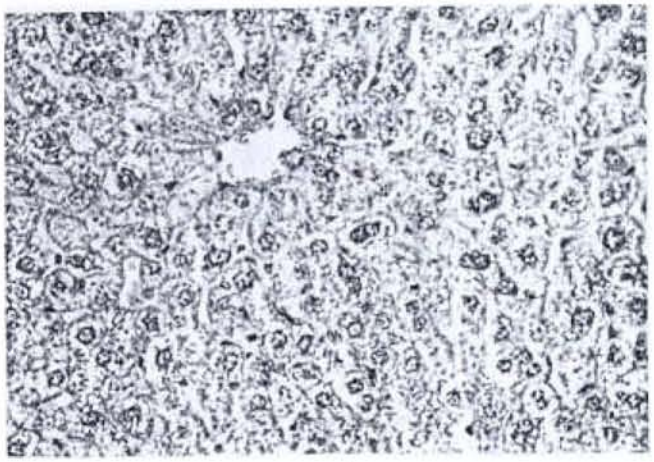


(b)

Figure 1. Lung of mice treated with SM (1 LD_{50}) after three days: (a) shows congestion and haemorrhage, H&E × 100 original; (b) as above in high power, H&E × 200 original.

4. DISCUSSION

The data presented deal primarily with the haematological, biochemical and histopathological changes induced by topically applied SM over a period of seven days. Topically applied SM caused a mild to moderate haemoconcentration (Table 1) probably due to the fluid loss from the vascular compartment as evidenced by the mild to moderate congestion of the viscera[13]. The observed decrease in WBC count (Table1) and plasma globulins [difference of total plasma proteins and albumin (Table 3) reflects the impairment of the immune system due to] SM intoxication. The occurrence of leukopenia was also observed in Iran-Iraq war victims exposed to SM[14]. Recently, Venkateswaran[15] have reported a



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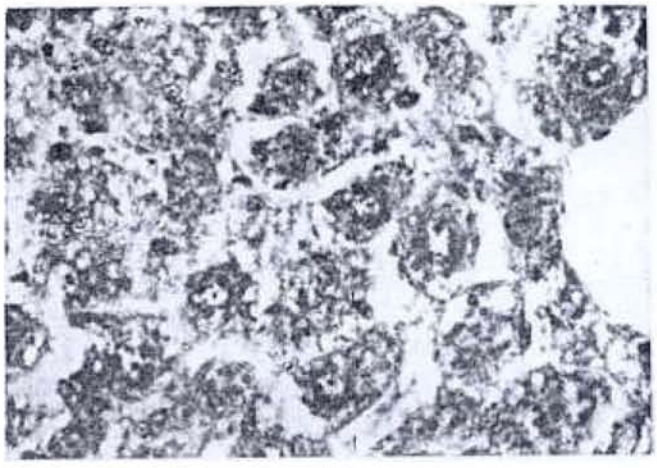
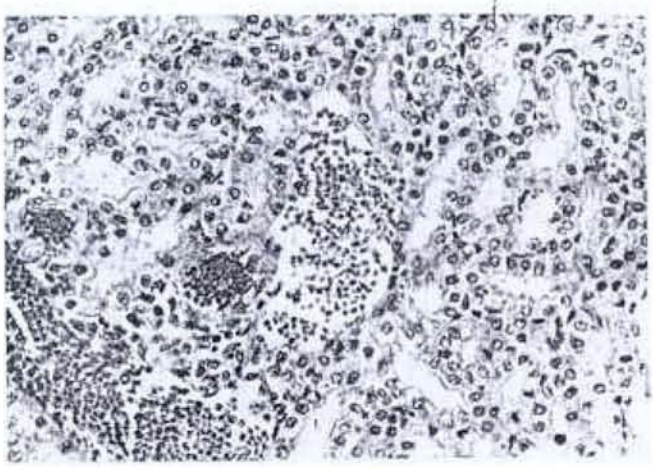
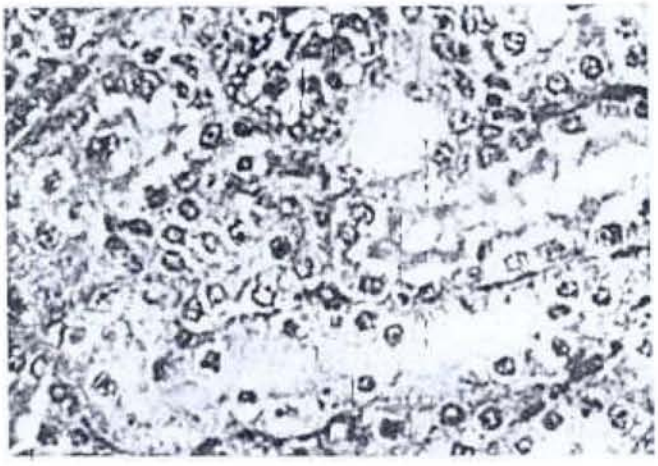


(b)

Figure 2. Liver of mice treated with SM (1.0 LD_{50}) after three days: (a) shows granulovacuolar degeneration of centrilobular hepatocytes, H&E × 100 original; (b) shows perinuclear clumping of cytoplasm and obliteration of chromatin material and necrotic hepatocytes, H&E × 200 original.



(a)



(b)

Figure 3. Kidney of mice treated with SM (1.0 LD_{50}) after three days: (a) shows congestion and haemorrhage, H&E × 100 original; (b) shows moderate degeneration and sloughing off renal epithelium, H&E × 200 original.

significant reduction in the cellularity of spleen and thymus revealing the immunotoxic effects of SM.

SM intoxication resulted in a coagulation defect leading to prolonged bleeding time, prothrombin time and clotting time. The cessation of bleeding occurs due to the formation of white thrombus or platelet plug composed of both platelets and loosely held fibrin monomers at the site of injury. The observed prolongation of the bleeding time without any appreciable change in the platelet count can be attributed to the probable decrease in the fibrinogen and prothrombin content due to the liver damage (Fig. 2) induced by SM intoxication. Further, the observed significant decrease in globulin fraction (difference of plasma total protein and albumin) in SM-intoxicated mice (Table 3) supports the above reasoning. The same factors are responsible for the observed prolongation of prothrombin time and clotting time (Table 2).

The observed simultaneous increases in plasma urea, uric acid and cholesterol can be attributed partially to the dehydration effect[13] as evidenced by haemo- concentration (Table 1). However, the role of renal factors cannot be ruled out, because kidneys were found to be affected (Fig. 3). Moreover, during an earlier study pertaining to various urinary metabolites[16], urine analysis showed a decreased output and presence of albumin (Combur-8-test of Boehringer Mannheim, FRG) in SM-intoxicated mice as compared to the control group (unpublished results). The increase in plasma uric acid might have

also originated from increased catabolism of purine nucleotide due to alkylation of DNA by SM14. Topically applied SM led to liver damage as there were significant increase in prothrombin time (Table 2) and decrease in plasma albumin and increase in plasma enzymes ALP, LDH and AST activities (Table 3). Histopathological observations of the liver sections (Fig. 2) provided evidence for SM-induced liver damage. Additionally, the observed increase in plasma enzymes, such as LDH, AST and CPK (Table 3) revealed the general state of illness after SM application.

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