EFFICACY OF A DCBRN-01VN SKIN DECONTAMINANT ON ANIMALS EXPOSED TO 2-CHLOROETHYL ETHYL SULFIDE

Ngoc Doan Vu[1,*], Quang Hung Vu[2], Thanh Vinh Nguyen[1], Ba Cuong Nguyen[1]

[1]*Faculty of Physics and Chemical Engineering, Le Quy Don Technical University, Hanoi, Vietnam*

[2]*Institute of Chemistry and Material, Academy of Military Science and Technology*

Abstract

Chemical warfare agents are an actual threat and decontamination for victims is a main concern when mass exposure occurs. The study evaluated the effectiveness of the skin decontamination agent DCBRN-01VN based on the oxime compound KBDO for CEES (2-chloroethyl ethyl sulfide). HPLC analysis method was used to determine the temperature after decontamination *in vitro* and *in vivo* tests and then to check biochemical and hematological indicators in rats. The test results show that DCBRN-01VN has a high efficiency of CEES decontamination, *in vitro*: with the ratio of CEES: decontaminant 1:20 in 10 minutes reaching 55.04%, with the ratio 1:10 in 20 minutes reached 55.95%, in 30 minutes reached 82.93%, in 60 minutes reached nearly 100%; *in vivo* test with a ratio of 1:15 in 20 minutes reached almost 100%. Results of biochemical and hematological examination showed no difference between the negative control group and two experimental groups (with decontamination and without decontamination) $P_{(2-1)} > 0.05$; $P_{(3-1)} > 0.05$. Histopathological analysis showed that decontamination also reduced damage caused by CEES.

Keywords: *Decontamination; DCBRN-01VN; sulfur mustard; CEES.*

1. Introduction

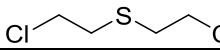
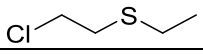
The potential use of chemical warfare agents on the battlefield is a continual threat to the soldier. Therefore, ensuring they are deployed with the most effective countermeasures is important to preserve life and limit adverse health effects [1]. Despite international regulation through the Chemical Weapons Convention, recent history has shown that military or civilian exposure to chemical warfare agents (CWA) can still occur. Among CWA, sulfur mustard (SM or HD) is one of the most famous, as it was massively used during World War I. It is a highly reactive agent that quickly penetrates skin [2] and alkylates numerous molecules, including DNA [3, 4].

Mustard gas (HD), a toxic chemical warfare agent, rapidly causes erythema, edema, and blistering after short skin exposure. 2-chloroethyl ethyl sulfide (CEES) is a surrogate of a mustard gas that possesses the same functional group (SCH_2CH_2Cl) as HD [5],

* Email: doanvn@lqdtu.edu.vn
DOI: 10.56651/lqdtu.jst.v1.n02.711.pce

which is responsible for the alkylation of proteins and therefore simulates its toxicological effects. HD and CEES have the sulfur and chlorine functionalities separated by a two-carbon chain; the only difference is that the real agent HD has an additional chlorine atom than the simulant. The related chemical structures make CEES an ideal simulant from a chemistry perspective; and offers similar chemical reactivity and similar surface tension. These similarities suggest that CEES should have similar contact with the surface on which the residual chemical resides as well as similar degradation pathways are present [6].

Table 1. Physicochemical properties and chemical structures for SM and simulants CEES

	Sulfur mustard (SM) CAS 505-60-2	**2-Chloroethyl ethyl sulfide (CEES) CAS 693-07-2**
Molecular mass ($g.mol^{-1}$)	159.08	124.63
Vapour pressure (Pa, at 25°C)	14.1-14.7	453
Density (at 25°C)	1.27 mg/L	1.07 g/mL
LD_{50} Oral - Rat	17 mg/kg	252 mg/kg[a]
Vapour density	5.4-5.6	4.3[a]
Log $K_{o/w}$	2.41-2.55	2.2[b]
Solubility	Poor in water (684 mg/L at 25°C). Good in organic solvents (e.g., alcohol, ether). Soluble in fat	Poor in water (1062 mg/L at 25°C)[b]. Good in organic solvents (e.g., alcohol, ether). Soluble in fat
Chemical structures		

Data from [3, 7].

[a]Fisher Material Safety Data Sheet.

[b]Estimated with EPISuite v4.11 ©2000e2012 EPA

SM is a vesicant agent; currently, no antidote is effective against SM poisoning. Therefore, timely removal of SM from contaminated skin after exposure is the only effective way to prevent or decrease tissue damage [8]. As a result, identifying an effective, non-toxic decontaminant has been the subject of research interest worldwide. Although efficient means of SM decontamination are available, several disadvantages limit their application [7]. To be of practical use under field conditions, any product in the form of a lotion that is to be used for decontamination must possess specific desirable properties [9]. First, it must be effective against all three types of SM agents. Second, for use as a skin decontaminant, it must be compatible with human skin and not cause any adverse reactions, at least over a limited period. These two criteria are responsible for the exclusion of many of the currently known decontamination systems because they contain very powerful reagents that damage the treated skin surface. Some of these systems are very alkaline, some use active chlorine or bleach solutions, and some use adsorbents associated with physical absorption. The

unchanged agents are highly toxic and must be carefully disposed of after the decontamination process. Thus, although these systems are, more or less, effective as decontaminants for equipment that has been exposed to SM, they are not applicable in the treatment of humans or animals exposed to SM [10].

Currently, countries have developed kits to decontaminate chemical and biological agents. Typical chemical agent decontamination kits are the M291, M258, M258A1, M58A1, M295, and CRS15 sets from the US and NATO, and the RSDL kit developed by Canada and NATO. RSDL contains Dekon 139 and a small amount of 2,3-butanedione monoxime (DAM). These compounds are dissolved in a solvent composed of polyethylene glycol monomethyl ether (MPEG) and water.

The new Kit lotion was synthesized in Le Quy Don laboratory containing potassium 2,3-butanedione monoxime (KBDO) in mono methoxy polyethylene glycol 550 (MPEG 550) with the sponge [11]. This solvent system is particularly important as it promotes the decontamination reaction by actively desorbing, retaining and sequestering the chemical agent while the active ingredient (KBDO) chemically reacts with and rapidly neutralizes the vesicant chemical or the organophophosphorous nerve agent. This reaction starts immediately, and neutralization is usually complete within two minutes. The current study was undertaken to determine the DCBRN-01VN solution reactivity against vesicant agent stimulant HD: 2-chloroethyl ethyl sulfide (CEES). The investigation used the HPLC analysis method to evaluate the decontamination efficiency *in vitro* and *in vivo* on pig skin; the test evaluates some biochemical and hematological parameters of rats in groups of non-infectious, non-decontaminated and decontaminated rats.

2. Experiment

2.1. Animals, chemicals, equipment

2.1.1. Chemicals

Reagents were purchased from commercial suppliers and used as received. DCBRN-01VN is a lotion containing potassium 2,3-butanedione monoxime (KBDO, was prepared in the laboratory of Le Quy Don Technical University) in mono methoxy polyethylene glycol 550 (MPEG 550, Merck) with 0.1722g KBDO per mL) [11]. Pure CEES solution (> 97%) was supplied by Sigma; formic acid for liquid chromatography and chromatographic solvents, CH_2Cl_2, MeCN, MeOH and acetic acid were supplied by Merck.

The DCBRN-01VN decontamination agent is reconstituted with a KBDO concentration of 1.25M with auxiliary components.

2.1.2. Equipments

HPLC high-pressure liquid chromatography system, Model: Series 222 (200),

Manufacturer: Perkin Elmer, USA; HPLC column C18, 3 µm, 33x4.6 mm; Vepl heating magnetic stirrer, Italy; Vortex 3000 shaker, USA; 4-Digit analytical balances OHAUS, USA; Technical balance Sartorius practum 612-1S, Germany; 40KHz ultrasonic cleaner, China.

Reaction flask: Transparent glass vial with a tight-fitting lid, Volumetric flask, Pipette, micropipette.

Table 2. The gradient program

Entry	Time (min)	Flow	Rate (mL/min)			
			%A	%B	%C	%D
1	0.50	1.00	60.0	40.0	0.0	0.0
3	10.00	0.50	60.0	40.0	0.0	0.0

The mobile phase was composed of a mixture Solvent A: Water + 0.1% HCOOH; Solvent B: MeCN + 0.1% HCOOH; Low-pressure limit: 0.000 psi; High-pressure limit: 6100 psi; Standby flow: 0.10 mL/min; Sampling rate: 2.2727 pts/s.

2.2. Standard curve for CEES

The CEES calibration curve consists of a series of standard solutions of mg/L concentration prepared from pure CEES solution in MeCN. 100 µL CEES (107 mg) diluted in 1L to obtain 107 mg/L CEES stock solution. Prepare a CEES calibration curve at concentrations of 35.67 mg/L; 53.50 mg/L; 71.33 mg/L; 107.0 mg/L, and 160.0 mg/L. The standard curve was drawn by plotting the peak area (y-axis) versus concentration (x-axis). Linear regression in Origin was performed (Y = A + BX), where A is the intercept, and B is the slope.

2.3. In vitro testing

The experiment was conducted in a 10 mL glass vial with a tight PE cap. Place the glass vial on the magnetic stirrer by taking 500 µL of CEES solution (concentration 10.700 mg/L in MeOH) into pre-made glass vials.

2.3.1. Effect of CEES: Decontaminant ratio

DCBRN-01VN decontamination solution with the calculated ratio was poured into the glass vial containing the CEES and performed the reaction for 10 minutes. The reaction was finished with 100 µL of acetic acid solution. Add MeCN and make up to 2 mL of the post-reaction solution. The sample solution was diluted 20 times with MeCN, filtered and analysed by HPLC.

Table 3. The volume of reagents in the experiment

CEES:DCBRN-01VN ratio	$V_{CEES/MeOH}$ µL	$V_{DCBRN-01VN}$ µL	V_{acid} µL	V_{MeCN} µL
1:10	500	345	100	1055
1:15	500	517.5	150	832.5
1:20	500	690	150	660

2.3.2. Effect of reaction time

DCBRN-01VN decontamination solution (ratio of simulant : decontaminant = 1:10 - the amount of decontamination agent 345 µL respectively) was poured into a glass vial containing the CEES and performed the reaction at 5, 10, 20, 30 and 60 min. The reaction was finished with 100 µL of acetic acid solution. Add MeCN and make up to 2 mL of the post-reaction solution. The sample solution was diluted 20 times with MeCN, filtered and analysed by HPLC.

2.4. In vivo testing

The experiment was conducted on a pig skin sample measuring 5 × 5 cm. Test ratio of CEES treatment with decontaminant at 1:10; reaction time is 10 minutes. Drip 50 µL of pure CEES solution into the center of the pig skin (marked area) and leave it for 3 to 5 minutes to allow the solution to penetrate the skin. Drip 3.43 mL of test solution DCBRN-01VN onto the spot where CEES was applied. Use a small glass rod to evenly disperse the treatment material solution and the CEES solution and allow it to stand for 5 minutes. After the reaction time, wipe the surface of the pig skin with a sponge, rinse the skin surface with distilled water (50 mL), place the entire sample in a Petri dish, and add CH_2Cl_2 so that the sample is submerged in the solvent. Obtain the sample solution after treatment for 15 - 20 minutes of ultrasonic extraction at room temperature. The sample solution was diluted 40 times with MeCN, filtered and analysed by HPLC.

2.5. Test and evaluate biochemical parameters of rats.

2.5.1. Animal

- Adult white rat (8 - 10 weeks old), both breeds, healthy, female has never given birth and not pregnant.

- Number of rats: 15 rats divided into 3 groups

+ Group 1: 5 rats (negative control) that did not apply the drug or decontaminate.

+ Group 2: 5 rats (positive control) with non-decontamination toxicity

+ Group 3: 5 toxic rats (tested) with decontamination with DCBRN-01VN

- Origin: Livestock Department - Central Institute for Drug Testing

- Weight: 180 - 220 g of each rat is not outside ± 20% of the average weight

- Quantity: 15 pcs

- Rats were kept under experimental conditions for at least 5 days before testing

- Condition of care: Temperature: 25 ± 3^oC

Relative humidity: 40 - 70%.

2.5.2. Test instruction

a) Sample preparation

Irritant sample: Use prototype

Decontamination Sample: Using Prototype

b) Prepare experimental animals

24 hours before the test day, an area of 3 × 3 cm was shaved (to the skin) on the back of each rat. Use a pen to mark the area of skin to be tested for contamination. Use only rats whose skin is not reddened.

c) Instruction

Step 1: Apply 100 µL of concentrated CEES to the marked skin and spread it evenly over the skin so that the solution is evenly spread over the marked skin (10 rats - groups 2 and 3). Group 1 (negative control) did not perform infection.

Step 2: For non-decontaminated animal samples (5 rats - group 2). Leave the CEES solution on the animal skin.

Step 3: For decontaminated animals (5 rats - group 3). After instilling 100 µl of CEES solution onto animal skin. Leave for 5 - 7 minutes for the substance to contact the skin. Next, add 6.9 mL of DCBRN-01VN solution to the infected skin, spread the solution evenly, and let the solution stay on the skin for 5 - 10 minutes. Then, rinse the skin with clean water using a sponge or cotton pad to wipe the test area.

2.5.3. Observation criteria

- Observation time: 72 hours.

- Observe the whole-body manifestations: ability to eat, exercise, abnormal symptoms.

- Observe and photograph the animal skin at 1 hour, 6 hours, 24 hours, 48 hours, and 72 hours after the test.

- Monitor the weight of rats immediately before the test and after the test.

- At the end of the test, all rats in 3 groups were taken blood for biochemical and hematological tests.

Student's t-test ($P < 0.05$) was applied to check whether the decontamination agent DCBRN-01VN significantly changed rats' weight and hematological and biochemical indices.

The tests were conducted at the Central Drug Testing Institute/Ministry of Health.

3. Results and discussion

3.1. Standard curve for determining CEES concentration

The standard curve is built based on the relationship between peak area and CEES concentration; the resulting equation was Y = 4820.7X – 13742 (R^2 = 0.993) (Fig. 1).

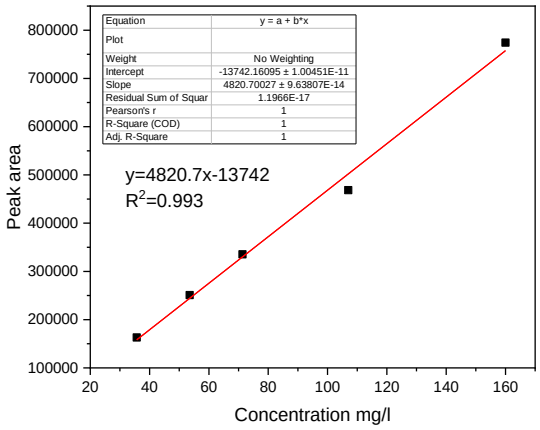


Fig. 1. Standard curve for CEES.

3.2. Evaluation of CEES decontamination efficiency by in vitro method

3.2.1. Effect of CEES: Decontamination ratio

The results of CEES treatment of DCBRN-01VN solution in the ratio of 1:10, 1:15 and 1:20 in 10 minutes are presented in Fig. 2.

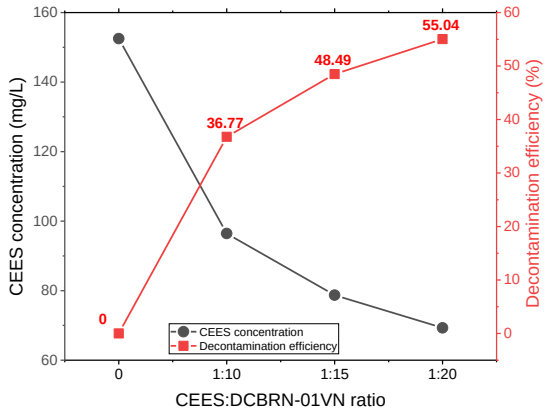


Fig. 2. Effect of ratio on CEES decontamination efficiency.

The results show the efficiency of CEES treatment increases with the ratio of decontaminant: CEES, with the highest processing efficiency of only 55.04% with a 20:1 ratio. The ability to degrade CEES by DCBRN-01VN solution was not apparent at the reaction time of 10 minutes. To give a more comprehensive view, we investigated the effect of reaction time on CEES decontamination efficiency.

3.2.2. Effect of reaction time

The results of processing CEES samples of DCBRN-01VN solution at the ratio 1:10 in 5; 10; 20; 30 and 60 minutes are presented in Fig. 3.

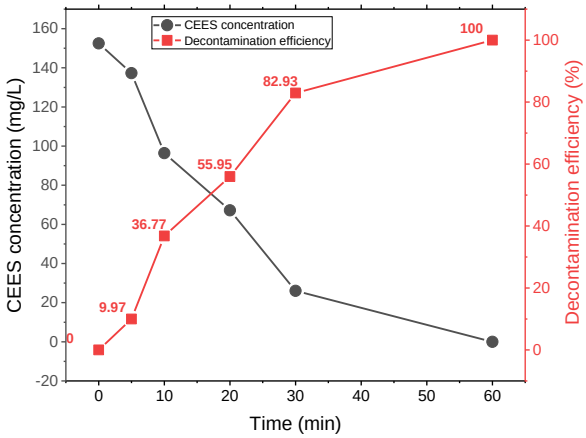


Fig. 3. Effect of time on CEES decontamination efficiency.

CEES treatment efficiency with a 1:10 reaction ratio gradually increased with reaction time. With an optimal ratio of 1:10, the DCBRN-01VN solution reached the highest processing efficiency of 100% in 60 minutes. The use of KBDO, a chemical decontaminant, is a rapid and effective method for skin decontamination via neutralization of chemical contaminants. As a decontaminant, potassium ketoxime is a non-irritant, and its degradation products are non-toxic [10]. Due to its desirable degradation properties, PBDO is appropriate for decontaminating contaminants *in vivo*.

3.3. Evaluation of CEES decontamination efficiency by in vivo method

The experiment was conducted on a pig skin sample measuring 4x5 cm. The ratio of CEES treatment with DCBRN-01VN solution at the ratio of 1:15 and 1:20, the reaction time is 20 minutes. The results of processing CEES samples of DCBRN-01VN solution at the ratios of 1:15 and 1:20 in 20 minutes are presented in Table 4.

Table 4. CEES treatment results of DCBRN-01VN solution in different ratios

Entry	Ratio CEES : DCBRN-01VN	CEES concentration after treatment (mg/L)	Decontamination efficiency %
1	-	150.1	-
2	1:15	Not detected	100
3	1:20	Not detected	100

The test results showed that the CEES removal efficiency of the DCBRN-01VN solution with the reaction ratio of 1:15 and 1:20 gave nearly 100% results after 20 minutes of reaction time. The explanation for this result is as follows: According to the *in vivo* method, in addition to the direct reaction of KBDO with CEES, mechanical and physical

removal methods also greatly support the decontamination efficiency. The high viscosity MPEG 550 solution quickly isolates CEES on contact, and the sponge's rapid absorption helps remove CEES quickly, leaving the remaining residue on the skin to be further decontaminated by the quantity of KBDO left over.

3.4. Test and evaluate biochemical indicators of rats

When exposed to the skin, group H blister agents bind to tissue proteins and cause characteristic skin changes such as erythema, hyperpigmentation, burns, epidermal destruction, and irreversible skin necrosis. 2-chloroethyl-ethylsulfide (CEES), with a similar structure and pathogenic mechanism to HD (edema, inflammation, cell death) but lower toxicity, has been identified as the most suitable surrogate for assays in the laboratory. We selected white rats with CEES poison as the study object, using the DCBRN-01VN decontamination solution. The test was conducted on three groups: negative control (non-infectious), positive control (toxic without decontamination) and test group (toxic with decontamination) at the Central Institute for Drug Testing/Ministry of Health; the test numbers include skin observations, hematological and biochemical indicators, etc.

The decontamination mechanism of KBDO for CEES is similar to that for sulfur mustard given in the investigation [12]; we propose the reaction mechanism of CEES with KBDO (Fig. 4). The nucleophilic substitution reaction between KBDO and CEES results in nontoxic products, unlike many other decontamination oxidation reactions that form toxic by-products, such as mustard sulfone or mustard sulfoxide. This is why KBDO in DCBRN-01VN decontamination solution does not cause corrosion or irritation, as strong oxidants do, and thus why it is appropriate for skin decontamination.

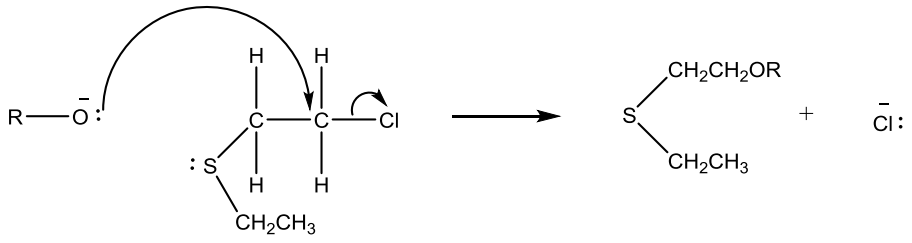


Fig. 4. Mechanism reaction of CEES with KBDO.

3.4.1. Systemic manifestations

The results of observation and photography of rats at 6, 24, 48 and 72 hours after testing are shown in Table 5.

Table 5. Systemic manifestations observed in 2 experimental and control groups

Group	Number of animals	Time (hours)			
		6	24	48	72
1	5	0	0	0	0
2	5	3T	2T	2T	2T
3	5	2T	1T	0	0

Note:

0 = No abnormality.

T = Expression of fatigue, decreased activity.

D = Dead.

- Test group 1 (negative control): Rats eat and drink normally.

- Test group 2 (not decontamination): At 6 hours, 3/5 rats showed signs of fatigue and reduced movement; up to 24, 48 and 72 hours, 2/5 rats showed signs of fatigue and decreased activity.

- Test group 3 (decontamination): At 6 hours, 2/5 rats showed signs of fatigue decreased activity; up to 24 hours, 1/5 of the rats showed signs of fatigue and reduced movement; At 48 hours, 72 hours, rats ate and acted normally. Test results on group 3 show the effectiveness of the decontamination process compared to group 2 (not decontaminated).

3.4.2. Monitoring the weight of rat

The results of weight monitoring of rats immediately before and after the test are presented in Table 6.

Table 6. Weight of rats before and after the test

Group	Before the test	After the test	P
1	196.20 ± 13.03	208.60 ± 15.82	$P_{before-after} > 0.05$
% compared to before the test		107.4	
2	204.60 ± 13.52	207.03 ± 13.41	$P_{before-after} > 0.05$
% compared to before the test		101.2	$P_{before(2-1)} > 0.05$ $P_{after(2-1)} > 0.05$
3	207.40 ± 9.71	207.45 ± 16.20	$P_{before-after} > 0.05$
% compared to before the test		100.0	$P_{before(3-1)} > 0.05$ $P_{after(3-1)} > 0.05$

Monitoring the weight of rats during the test showed:

- Before the test: The average weight of rats in the test groups before the test did not differ from the control group ($P_{before\ (2-1)} > 0.05$; $P_{before\ (3-1)} > 0.05$).

- After 72 hours: The rat in the negative control group gained weight after 72 hours of the test. The rats in the 2nd and 3rd groups almost did not gain weight compared to before the experiment. There was no significant difference in the weight of the rats when comparing the 72 hours after-test with the before-test in each group (before-after $P > 0.05$). There was no significant difference in mean weight between the experimental group and the control group after the test ($P_{after\ (2-1)} > 0.05$; $P_{after\ (3-1)} > 0.05$).

3.4.3. Hematological indicators

The test results of hematological indicators of the 2 experimental groups compared with the negative control group after the test are presented in Table 7.

Table 7. Comparison of hematological indicators of the two experimental groups compared with the negative control group

	Group 1	**Group 2**	**P (2-1)**	**Group 3**	**P (3-1)**
Erythrocyte ($\times 10^{12}$/l)	7.6 ± 0.9	6.4 ± 1.2	> 0.05	6.7 ± 0.5	> 0.05
Leucocyte ($\times 10^{9}$/l)	12.5 ± 2.2	9.4 ± 2.3	> 0.05	9.9 ± 2.5	> 0.05
Thrombocyte ($\times 10^{9}$/l)	716.3 ± 204.9	713.8 ± 431.9	> 0.05	690.6 ± 97.9	> 0.05
Hematocrit (%)	42.3 ± 4.1	36.9 ± 7.2	> 0.05	37.1 ± 3.0	> 0.05
Hemoglobin (g/dl)	13.1 ± 1.1	11.3 ± 2.3	> 0.05	11.8 ± 0.8	> 0.05

There was no significant difference in hematological indexes between the two experimental groups and the negative control group ($P_{(2-1)} > 0.05$; $P_{(3-1)} > 0.05$), proving that the decontaminant does not significantly affect the hematological indicators of rats.

3.4.4. Biochemical indicators in blood

The test results of biochemical indicators in blood of the 2 experimental groups compared with the negative control group after the test are presented in Table 8.

Table 8. Results of comparison of biochemical indicators of 2 experimental groups compared with the negative control group

Indicator	Group 1	Group 2	$P_{(2-1)}$	Group 3	$P_{(3-1)}$
AST (U/l)	80.5 ± 8.0	61.0 ± 17.2	> 0.05	73.1 ± 8.3	> 0.05
ALT (U/l)	24.7 ± 7.1	24.7 ± 9.1	> 0.05	19.8 ± 7.4	> 0.05
Total bilirubin (μmol/l)	2.6 ± 0.2	2.5 ± 1.4	> 0.05	4.4 ± 4.9	> 0.05
Total protein (g/l)	**69.4 ± 0.9**	**60.1 ± 2.6**	**< 0.05**	**60.4 ± 3.3**	**< 0.05**
Cholesterol (mmol/l)	**1.8 ± 0.2**	**1.3 ± 0.1**	**< 0.05**	**1.2 ± 0.2**	**< 0.05**
Urea (mmol/l)	**4.5 ± 0.6**	**2.9 ± 1.3**	**< 0.05**	**3.0 ± 0.8**	**< 0.05**
Creatinine (μmol/l)	55.3 ± 10.5	41.8 ± 4.0	< 0.05	47.5 ± 5.0	> 0.05
Glucose (mmol/l)	7.8 ± 2.0	6.9 ± 0.6	> 0.05	6.8 ± 0.9	> 0.05

There are three significant difference indexes of protein, total cholesterol and urea of the 2 test groups compared with the negative control group ($P_{(2-1)} < 0.05$; $P_{(3-1)} < 0.05$). In the 2nd test group (without decontamination), there was a statistically significant low creatinine index compared with the negative control group ($P < 0.05$). The other indices did not have significant differences between the two experimental and negative control groups ($P_{(2-1)} > 0.05$; $P_{(3-1)} > 0.05$).

3.4.5. Skin manifestations

The results of observations and photographs of rats at 6, 24, 48 and 72 hours after testing can be seen in Table 9.

- Test group 1 (negative control): Normal rat skin.

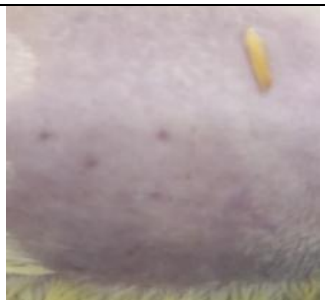
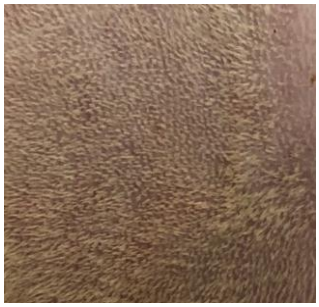
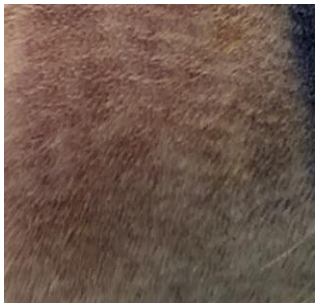
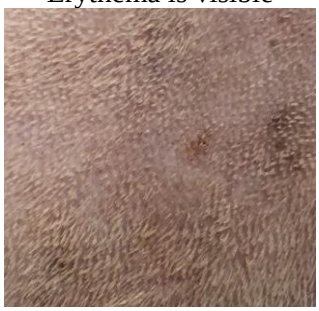
- Test group 2 (not decontamination): At 6 hours, the rat's skin showed signs of severe redness, and some bruises; up to 24, 44 and 72 hours, the rat's skin was darkened.

- Test group 3 (decontamination): At 6 hours, the rat's skin showed signs of mild erythema. By 24, 48 and 72 hours, erythema was visible.

In the pathology of SM toxicity, the duration of contact with the skin and SM concentration play important roles in diffusion of the chemical through the skin. In humans, the first signs of morphologic changes include the appearance of erythema followed by edema formation. The extent of these effects is dose-dependent. It is rather

difficult to extrapolate doses from rat to human, but the dose applied in our experiment, which was able to induce a severe reaction.

Table 9. Observational manifestations on rat skin of 2 test and control groups

	Observable manifestations		
	Group 1	**Group 2**	**Group 3**
6h	 Normal	 Red and bruised skin	 Mild erythema
24h	 Normal	 Dark skin	 Erythema is visible
48h	 Normal	 Dark skin	 Erythema is visible
72h	 Normal	 Dark skin	 Erythema is visible

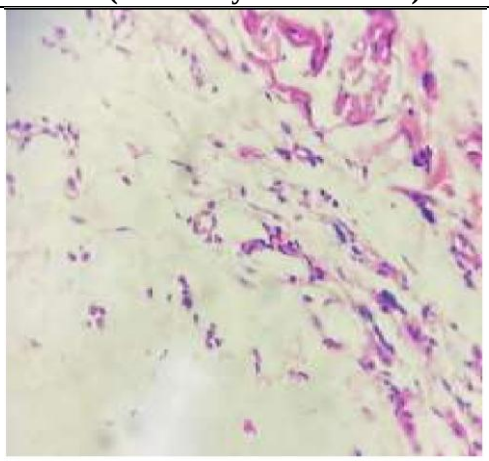
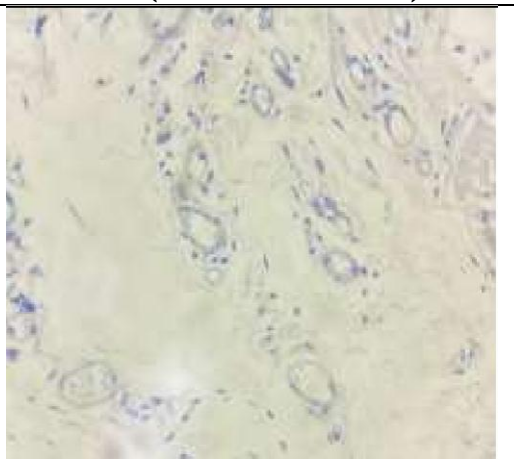
The inflammatory process was maximal between 24 - 72 hours post-exposure to CEES. In the present study, KBDO proved to be an efficient decontaminant for reducing the morphologic changes induced by CEES, specifically the appearance of dark skin. Our results show that decontamination with KBDO after CEES exposure in rats delayed the appearance of mild erythema skin formation by at least 6 hours, whereas in the control group that was not treated with decontaminant, red and bruised skin was observed after exposure to CEES. These findings can be explained as follows: CEES penetrated into the skin, and some CEES had entered the skin before decontaminant was applied. The control decontaminant with no active ingredient failed to decompose CEES within the skin, resulting in apparent skin damage. It is well known that the earlier a decontaminant is applied, the more effective it will be.

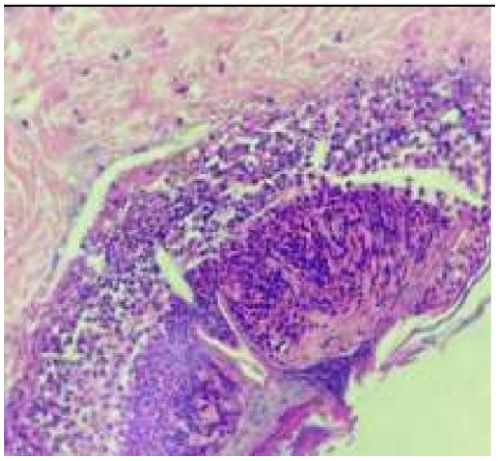
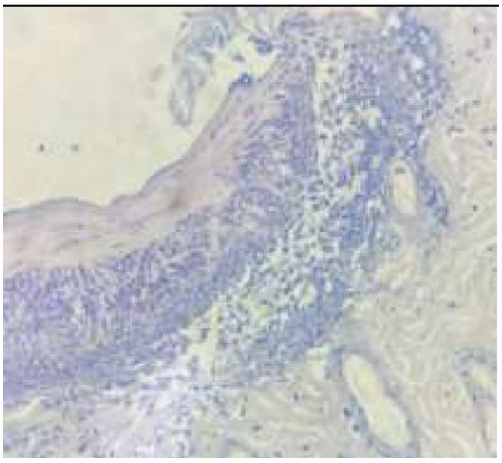
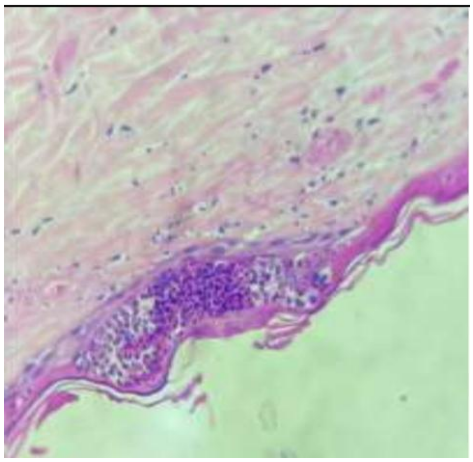
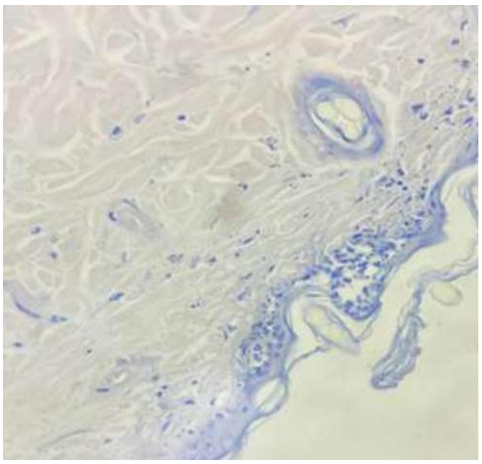
3.4.6. Histopathological analysis

To assess the extent of cell damage, rat skin specimens of the test groups were used for epidermal analysis. The results of the analysis are shown in the following Table 10.

The microscopic observation of the experimental rat skin showed that in the negative control group, the skin structure of the rat was normal. In the non-decontaminated test group, severe skin lesions appeared, but the level was not too severe. This is appropriate because the delay in decontamination (after 5 min of exposure) resulted in significant toxin penetration and destruction of exposed skin cells. Thus, from the test results, it is recommended that decontamination be carried out as soon as possible after exposure. The end of decontamination requires continued medical support and treatment.

Table 10. Histopathological analysis

Group	HE (Hematoxylin and Eosin)	PAS (Periodic Acid-Schiff)
1		

Group	HE (Hematoxylin and Eosin)	PAS (Periodic Acid-Schiff)
	Normal	
2		
	Dermatitis, chronic active stromal inflammation with atrophy of the skin	
3		
	Dermatitis, chronic active stromal inflammation with atrophy of the skin	

4. Conclusions

DCBRN-01VN decontamination solution has excellent CEES decontamination efficiency. *In vitro* test with a CEES: decontaminant with a ratio of 1:10 in 60 minutes reached nearly 100%; *in vivo* test with 1:15 in 20 minutes reached almost 100%. Biochemical and hematological examination results showed no difference between the negative control group and the two experimental groups, and histopathological analysis showed that decontamination also reduced the damage caused by CEES. The results of this study indicate that DCBRN-01VN reacted decontamination solution rapidly and entirely with CEES; thus, it was found to be a suitable and effective skin decontaminant against vesicants.

References

[1] Hazem Matar et al., "Development of a Next Generation Military Skin Decontaminant: Initial Efficacy Studies of Zirconium Hydroxide", *Dermal Absorption and Decontamination: A Comprehensive Guide*, 2022, pp. 169-181. DOI: 10.1007/978-3-031-09222-0_10

[2] R. P. Chilcott et al., "Human skin absorption of bis-2-(chloroethyl)sulphide (sulphur mustard) in vitro", *J Appl Toxicol*, Vol. 20(5), pp. 349-355, 2000 Sep-Oct. DOI: 10.1002/1099-1263(200009/10)20:5<349::AID-JAT713>3.0.CO;2-O

[3] M. P. Shakarjian et al., "Mechanisms Mediating the Vesicant Actions of Sulfur Mustard after Cutaneous Exposure", *Toxicological Sciences*, Vol. 114, No. 1, 2009, pp. 5-19. DOI: 10.1093/toxsci/kfp253

[4] M. Spiandore et al., "Efficacy of scalp hair decontamination following exposure to vapours of sulphur mustard simulants 2-chloroethyl ethyl sulphide and methyl salicylate", *Chemico-Biological Interactions*, Vol. 267, pp. 74-79, 2017. DOI: 10.1016/j.cbi.2016.07.018

[5] Ye, Feng, et al., "Chemical safety concerns of sulfur mustard analog, 2-chloroethyl ethyl sulfide, in laboratory study", *Annals of Military and Health Sciences Research*, Vol. 19(4), 2021. DOI: 10.5812/amh.121268

[6] Cao, Yachao, et al., "In vitro human skin permeation and decontamination of 2-chloroethyl ethyl sulfide (CEES) using Dermal Decontamination Gel (DDGel) and Reactive Skin Decontamination Lotion (RSDL)", *Toxicology Letters*, Vol. 291, pp. 86-91, 2018. DOI: 10.1016/j.toxlet.2018.04.015

[7] Ganesan, K, et al., "Chemical warfare agents", *J Pharm Bioallied Sci*, Vol. 2, 166, 2010. DOI: 10.4103/0975-7406.68498

[8] Centers for Disease Control and Prevention, "Guidelines for safe work practices in human and animal medical diagnostic laboratories". Recommendations of a CDC-convened biosafety blue ribbon panel. Morbidity and Mortality Weekly Report (MMWR), 61, 1-103, 2012.

[9] Sun, Jing-Hai, et al., "Skin decontamination efficacy of potassium ketoxime on rabbits exposed to sulfur mustard", *Cutaneous and Ocular Toxicology*, Vol. 34, No. 1, pp. 1-6, 2015. DOI: 10.3109/15569527.2014.880454

[10] Jr. Braue, H. Ernest et al., "Efficacy studies of reactive skin decontamination lotion, M291 skin decontamination kit, 0.5% bleach, 1% soapy water, and skin exposure reduction paste against chemical warfare agents, Part 1: Guinea pigs challenged with VX", *Cutaneous and Ocular Toxicology*, Vol. 30, No. 1, pp. 15-28, 2011. DOI: 10.3109/15569527.2010.515280

[11] Vu Ngoc Doan et al., "Investigation of efficacy of a DCBRN-01VN skin decontaminant against organophospate agent", *Journal of Science Technique - Section on Physics and Chemical Engineering*, Vol. 1, No. 01, pp. 110-121, 2023. DOI: 10.56651/lqdtu.jst.v1.n01.634.pce

[12] Vũ Ngọc Doãn, Đoàn Hữu Hùng, Nguyễn Thành Vinh, "Tổng hợp và khảo sát khả năng tiêu độc của KBDO đối với chất độc yperit," *Tạp chí Khoa học và Kỹ thuật*, Vol. 17, No. 02, tr. 25-34, 2022. DOI: 10.56651/lqdtu.jst.v17.n02.306

HIỆU QUẢ CỦA CHẤT KHỬ NHIỄM DA DCBRN-01VN ĐỐI VỚI ĐỘNG VẬT TIẾP XÚC VỚI CHẤT 2-CHLOROETHYL ETHYL SULFIDE

Vũ Ngọc Doãn[a], Vũ Quang Hưng[b], Nguyễn Thành Vinh[a], Nguyễn Bá Cường[a]

[a]*Khoa Hóa - Lý kỹ thuật, Trường Đại học Kỹ thuật Lê Quý Đôn*

[b]*Viện Hóa học - Vật liệu, Viện Khoa học và Công nghệ quân sự*

Tóm tắt: Các tác nhân chiến tranh hóa học là một mối đe dọa thực sự và việc khử nhiễm cho nạn nhân là mối quan tâm chính khi xảy ra phơi nhiễm hàng loạt. Nghiên cứu tiến hành đánh giá hiệu quả chất khử nhiễm da DCBRN-01VN trên cơ sở hợp chất oxime KBDO đối với CEES (2-chloroethyl ethyl sulfide). Phương pháp phân tích HPLC được sử dụng để xác định nồng độ sau khử nhiễm trong các thử nghiệm *in vitro* và *in vivo*, sau đó kiểm tra các thông số sinh hóa, huyết học trên chuột. Kết quả thử nghiệm cho thấy DCBRN-01VN có hiệu quả khử nhiễm CEES cao, *in vitro*: với tỉ lệ CEES : chất khử nhiễm là tỉ lệ 1:20 trong thời gian 10 phút đạt 55,04%, với tỉ lệ 1:10 trong 20 phút đạt 55,95%, 30 phút đạt 82,93%, 60 đạt gần 100%; *in vivo* với tỉ lệ 1:15 trong 20 phút đạt gần 100%. Kết quả kiểm tra sinh hóa và huyết học không có thấy sự khác biệt giữa nhóm chứng âm và 2 nhóm thử nghiệm (có khử nhiễm và không khử nhiễm) $P_{(2-1)} > 0,05$; $P_{(3-1)} > 0,05$. Phân tích mô bệnh học cho thấy khử nhiễm cũng làm giảm tổn thương do CEES gây ra.

Từ khoá: *Khử nhiễm; DCBRN-01VN; chất độc hại da HD; CEES.*

Received: 05/09/2023; Revised: 03/10/2023; Accepted for publication: 17/11/2023

❑