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Evaluation of CC2 as a Decontaminant in Various Hydrophilic and Lipophilic Formulations Against Sulphur Mustard

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Objective To evaluate CC2 (N, N` -dichloro-bis [2, 4, 6-trichlorophenyl] urea) in various hydrophilic and lipophilic formulations as a personnel decontaminant for sulphur mustard (SM). **Methods** Twenty percent of CC2 was prepared as a suspension or ointment with various chemical agents and its stability was evaluated by active chlorine assay. The efficacy was evaluated in mice by recording the mortality after applying 29 LD_{50} of SM (LD_{50} = 8.1 mg/kg dermally) and decontaminating it after 2 min with 200 mg of the formulation. Studies were also carried out with 10% and 20% CC2 in acacia and hydroxypropyl cellulose, and the suspensions were stored in polyethylene containers. The stability of the suspensions was evaluated by active chlorine assay. The efficacy was evaluated by recording the mortality after applying 29 LD_{50} of SM in mice and 12 LD_{50} of SM in rats (LD_{50} = 2.4 mg/kg dermally), and decontaminating it with the formulations. LD_{50} by different routes and primary skin irritation test of CC2 were also carried out. **Results** CC2 reacted with peanut oil and neem oil, and was unstable in povidone iodine and Fuller's earth. Good stability was achieved with petroleum jelly, honey, polyvinyl pyrrolidone, calamine lotion, acacia and hydroxypropyl cellulose. Though CC2 was stable in lipophilic formulations, it did not protect the animals. The hydrophilic formulations particularly acacia and hydroxypropyl cellulose gave very good protection and was stable in the polyethylene containers for a period of 1 year. The efficacy of 20% CC2 was better than 10% CC2. The oral and dermal LD_{50} of CC2 was found to be above 5.0 g/kg. CC2 was also found to be nonirritant. **Conclusion** Twenty percent of CC2 in hydroxypropyl cellulose is better with respect to stability, efficacy and ease of decontamination. CC2 is also a safe chemical.

Key words: Sulphur mustard; Decontamination; CC2; Active chlorine; Formulations; Hydrophilic; Lipophilic; Acacia; Hydroxypropyl cellulose

INTRODUCTION

Bis (2-chloroethyl) sulphide, commonly known as sulphur mustard (SM) or mustard gas, is an alkylating agent that causes serious blisters upon contact with human skin. SM is frequently used as a chemical warfare agent and several reports are available of its recent use[1-4]. In spite of the successful implication of the Chemical Weapons Convention

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(CWC), SM being used clandestinely during war or by terrorist groups remains a threat, due to the simple method of its preparation. For personnel engaged in the destruction of SM and during inspections by the Organization for the Prohibition of Chemical Weapons (OPCW), a decontamination agent will be very useful.

SM forms sulphonium ion in the body and alkylates DNA leading to DNA strand breaks and cell death[5,6]. Due to high electrophilic property of the sulphonium ion, SM binds to a variety of cellular macromolecules and fatality may occur due to multi organ failure[7,8]. Eyes, skin and the respiratory tract are the principal target organs of SM toxicity[5,9]. Several antidotes have been reported for the systemic toxicity of SM in experimental animals[8,10 16].

All the antidotes so far screened have given only limited protection, and decontamination of SM, immediately after contact is recommended as the best protection[7]. The most commonly used decontaminant is Fuller's earth (known as montmorillonite, a native form of aluminium silicate) that removes SM by adsorption, thereby reducing the toxicity. SM is highly lipophilic and gets absorbed very quickly after contact with skin. Therefore, decontamination has to be done quickly to limit the absorption and skin injury. The authors earlier reported a combination of N, N'-dichloro-bis [2, 4, 6-trichlorophenyl] urea, known as CC2 and Fuller's earth, as a better decontaminant for SM by virtue of physical adsorption and chemical interaction[17,18]. As this formulation is a dry powder, decontamination will be difficult on larger areas of the body. A liquid preparation will be easy to use and decontaminate. The present study is aimed to evaluate the efficacy of CC2 in various hydrophilic and lipophilic formulations as a decontaminant for SM.

MATERIALS AND METHODS

Animals

Randomly bred adult Swiss female mice (25-30g body weight) and Wistar male rats (200-240g body weight) from DRDE (Defence Research and Development Establishment) animal house facility were used. They were housed in polypropylene cages (mice–five per cage; rats–four per cage) with dust free rice husk as bedding material, and were provided with food (Amruth India Ltd.) and water *ad libitum*. For primary skin irritation tests, randomly bred adult female albino rabbits (2.0-2.5 kg) from DRDE animal house facility were used. They were housed in wire mesh cages and were provided with food (Amruth India Ltd.) and water *ad libitum*. This study has the approval of the Establishment's Ethical Committee.

Chemicals

SM was synthesized in the chemistry laboratory and was found to be more than 99% pure by gas chromatographic analysis. CC2 was also synthesized in the chemistry laboratory and its active chlorine was checked by titrimetric method. Theoretically the active chlorine should be 14.5%. Fuller's earth, hydroxypropyl cellulose and polyvinyl pyrrolidone were purchased from Supra Chem (India), Acros (USA) and Fluka (USA) respectively. Others *viz.*, acacia i. p., honey, turmeric, silicone oil, etc., were purchased locally.

Formulations

CC2 was prepared as an ointment with petroleum jelly or silicone oil. With other agents it was prepared as a suspension by grinding all the constituents in a Retsch Grinder (Germany). The percentages of constituents of the ointments and the suspensions are given in Table 1. The ointments and suspensions were stored in stoppered glass test tubes.

TABLE 1

Formulations Used in This Study and Its Constituents

Abbreviation	Constituents (Percentage, by Weight)
FE-CC2	Fuller's earth (80%) and CC2 (20%)
PJ-CC2	Petroleum jelly (80%) and CC2 (20%)
SO-CC2	Silicone oil (80%) and CC2 (20%)
H-CC2[a]	Honey (80%) and CC2 (20%)
PI-CC2[a]	Povidone iodine ointment (80%) and CC2 (20%)
PP-CC2[a]	Polyvinyl pyrrolidone (20%), water (60%) and CC2 (20%)
CL-CC2[a]	Calamine lotion (80%) and CC2 (20%)
A-CC2[a]	Acacia i. p. (20%), water (60%) and CC2 (20%)
TP-CC2[a]	Turmeric powder (20%), water (60%) and CC2 (20%)
HC-CC2[a]	Hydroxypropyl cellulose (1%), water (79%) and CC2 (20%)

[a] Compatible with water.

Note. In A-CC2 and HC-CC2, formulations containing 10% CC2 were also prepared, in which the water percentage was increased by 10%.

Stability Studies of CC2

The stability of CC2 in various formulations was evaluated by the titrimetric method for active chlorine by using standard sodium thiosulphate solution, and starch-iodine as indicator. The active chlorine was converted into percentage. The stability of the formulations was checked for a period of 1 year.

Decontamination Studies in Mice for an Effective Formulation

Hair from the back of mice was closely clipped using a pair of curved scissors, 24 h prior to the experiment. 5 µl of SM was applied on the back with the help of an autopipette. The dose applied was 232 mg/kg equivalent to 29 LD_{50} (LD_{50} of SM in mice = 8.1 mg/kg dermally, for 14 day observation). The applied SM was decontaminated after 2 min with the formulations. 200 mg of the formulation was smeared on the SM applied area and rubbed gently using a smooth glass rod, and the formulation was left on the skin of the animal. For each group 5 mice were used. After decontamination the mortality of the animals was observed for up to 14 days. The efficacy of the formulations was evaluated for 90 days.

Decontamination Studies in Mice and Rats for an Effective Formulation and Percentage of CC2

10% and 20% CC2 were prepared in acacia or hydroxypropyl cellulose and stored in polyethylene containers. Hair from the back of mice and rats was closely clipped using a pair of curved scissors, 24 h prior to the experiment. 5 µl of SM was applied on the back with the help of an autopipette. In mice the dose was 232 mg/kg equivalent to 29 LD_{50}

(LD_{50} of SM in mice = 8.1 mg/kg dermally, for 14 day observation). In rats the dose was 28.9 mg/kg equivalent to 12 LD_{50} (LD_{50} in rats = 2.4 mg/kg dermally; for 14 day observation). The applied SM was decontaminated after 2 min with the formulations. 200 mg of the formulation was smeared on the SM applied area and rubbed gently using a smooth glass rod, and the formulation was left on the skin of the animal. For each group 4 mice and 4 rats were used. After decontamination the mortality of the animals was observed for up to 14 days. The stability and efficacy of the formulations were evaluated for 1 year.

LD_{50} Determination

LD_{50} of CC2 was estimated through various routes for assessing safety of the chemical. The animals were fasted for 3 h before administration of the chemical. For oral LD_{50}, CC2 was prepared in acacia (20%), and for intraperitoneal and dermal LD_{50}, CC2 was prepared in hydroxypropyl cellulose (1%). For each route and sex 3 to 4 groups were used. The mortality of the animals was observed for 14 days. LD_{50} was estimated by Gad and Weil method[19]. For all LD_{50} determinations 3 to 4 groups were used with each group consisting of 4 animals.

Primary Skin Irritation Test

Primary skin irritation tests of FE-CC2, PJ-CC2, A-CC2 and HC-CC2 were carried out on rabbits. Hair on the back of the rabbits was closely clipped 24 h prior, and four areas (2.5 cm^2) were marked, two on the right side and two on the left side. The two areas on the left side were kept as control and on the right side 0.5 g of the agent was applied. The sites were then covered with a gauze patch and parafilm, and kept in position with adhesive tapes. For each sample 3 sites were used. The animals were immobilized in rabbit restrainer for 4 h, and the coverings were then removed and the areas were wiped with moist cotton. The test areas were inspected and evaluated for erythema, eschar and edema formation, and scored[20].

Statistical Analysis

The decontamination efficacy of the various agents was tested by Friedman repeated measures and analysis of variance on ranks with Dunnett's test. For this cumulative percent of death for each day for a period of 14 days was used. Body weights were analyzed by one way ANOVA followed by Student Newman Keuls multiple comparisons test. Sigma Stat (Jandel Scientific Corporation, USA) was used for the statistical analysis. A probability less than 0.05 was taken as statistically significant.

RESULTS

CC2 was found to be stable in petroleum jelly, honey, polyvinyl pyrrolidone, calamine lotion, acacia and hydroxypropyl cellulose for 3 months to 1 year. The active chlorine was between 85% to 100% (14.5% Cl_2 is equal to 100% CC2) during this period (Table 2). CC2 was not stable in peanut oil and neem oil, and reacted immediately. In povidone-iodine ointment, CC2 was stable for less than 1 day and the activity was about 10% afterwards. The active chlorine was also less in formulations with turmeric and

silicone oil. In the presence of Fuller's earth the activity of CC2 decreased over a period of time and was stabilized at about 50%.

TABLE 2

Active Chlorine (%) in Various Formulations Assayed at Different Times

Formulation	Days After Preparation						
	1	7	14	28	90	180	365
FE-CC2	99	92	80	50	55	50	46
PJ-CC2	100	100	99	100	98	105	-
SO-CC2	70	77	78	79	75	-	-
H-CC2	99	99	100	100	99	-	-
PI-CC2	100	10	12	6	9	-	-
PP-CC2	101	89	83	98	111	-	-
CL-CC2	107	93	90	84	99	-	-
A-CC2	89	89	85	84	88	98	98
TP-CC2	70	53	76	59	69	-	-
HC-CC2	100	98	95	97	96	97	96

Note. Each value is a mean of at least two assays.

The primary skin irritation test carried out in rabbits showed that CC2 was non-irritant in combination with Fuller's earth, petroleum jelly, acacia and hydroxypropyl cellulose (data not shown).

Initial experiments were carried out in mice with various formulations containing 20% CC2. All the mice that were applied 5 µl of SM without any decontamination died within 1 week. The 50th percentile mortality (median mortality), i. e., mice that died up to 7 days post SM application was 100% in undecontaminated group. The formulations without CC2, did not protect the mice. Significant protection and stability were achieved with PP-CC2, A-CC2 and HC-CC2 compared to Fuller's earth alone (FE), which is a conventional treatment. The 50th percentile mortality was either 0% or 20% over a period of 3 months in these formulations. Though the 50th percentile median mortality was less in FE-CC2, H-CC2, CL-CC2 and TP-CC2, the decontamination efficacy was not significant at all the post preparation times compared to FE only group (Table 3). PI-CC2 did not give significant protection compared to FE, but the protection was significant compared to SM only group. PJ-CC2 and SO-CC2, both are lipophilic in nature, did not protect the mice compared to SM only group. The 50th percentile mortality was 100% in PJ-CC2 and 60% in SO-CC2.

Since A-CC2 and HC-CC2 gave very good protection, 10% and 20% of CC2 were prepared by using these two agents. The prepared suspension was stored in a polyethylene container for storage stability and decontamination efficacy up to 1 year. Mice and rats applied with 5 µl SM and decontaminated with 0% CC2 (formulation only) died within 1 week. Both 10% and 20% CC2 in presence of acacia or hydroxypropyl cellulose gave significant protection as a decontaminant against dermally applied SM in mice up to 1year (Table 4). But in rats 20% CC2 only gave significant protection both in presence of acacia and hydroxypropyl cellulose. 10% CC2 gave protection only up to 3 months in presence of acacia and 6 months in presence of hydroxypropyl cellulose (Table 5). Estimation of active chlorine of 10% and 20% CC2 showed that both preparations were stable up to 1 year after preparation.

TABLE 3

Decontamination Efficacy of Various CC2 Formulations Against Sulphur Mustard (SM) in Mice

Formulation	Days After Preparation				
	1	7	14	28	90
SM only	100	(65,100)			
FE alone	20	(5,55)			
PJ alone	100	(80,100)			
H alone	100	(65,100)			
A alone	100	(60,100)			
PI alone	60	(5,100)			
PP alone	100	(45,100)			
HC alone	100	(80,100)			
FE-CC2	0 *	40	40	40	40
	(0,0)	(5,60)	(25,60)	(0,80)	(0, 75)
PJ-CC2	100	100	100	100	100
	(85,100)	(100,100)	(100,100)	(100,100)	(100, 100)
SO-CC2	60	60	40	60	80
	(25,60)	(25,60)	(40,40)	(60,60)	(80, 80)
H-CC2	0 *	20	40	40	20
	(0,35)	(5,60)	(0,95)	(5,40)	(0, 60)
PI-CC2	40	80	60	60	40
	(20,60)	(25,80)	(10,60)	(0,100)	(0, 100)
PP-CC2	0 *	0 *	0 *	0 *	20 *
	(0,0)	(0,0)	(0,20)	(0,0)	(0, 40)
CL-CC2	0 *	0 *	40	20	20
	(0,0)	(0,20)	(25,40)	(20,20)	(20, 20)
A-CC2	0 *	0 *	0 *	0 *	20 *
	(0,0)	(0,0)	(0,0)	(0,0)	(0, 35)
TP-CC2	20 *	0 *	40	20 *	20
	(5,20)	(0,30)	(25,60)	(0,35)	(5, 50)
HC-CC2	0 *	0 *	0 *	20 *	0 *
	(0,0)	(0, 0)	(0,0)	(0,20)	(0, 20)

$X^2 = 660.8$; $df = 57$; $P = < 0.001$

Note. Values are 50th percentile mortality i. e., mice died up to 7 days post SM application.

Figures in parentheses are 25th and 75th percentiles. Five mice were used for each formulation and day after preparation.

Dose of undiluted SM was 232 mg/kg (29 fold LD_{50}) applied dermally.

Dose of agents for decontamination was 200 mg smeared on the SM applied area.

*Statistically significant compared to FE group by Friedman's repeated measures ANOVA followed by Dunnett's method.

The safety of CC2 was assessed by using LD_{50} determination. The oral and dermal LD_{50} of CC2 was above 5.0 g/kg. Intraperitoneal LD_{50} in mice was 0.673 mg/kg and 0.800 mg/kg in male and female, and 0.951 mg/kg and 1.345 mg/kg in male and female rats (Table 6). The animals that were administered up to 6 400 mg/kg of CC2 orally, did not show any significant difference in body weight compared to the control group (Table 7). No abnormality was observed in general behaviour of the animals.

TABLE 4

Decontamination Efficacy of Various Percentages of A-CC2 and HC-CC2 Formulations Against Sulphur Mustard (SM) in Female Mice

Days after Preparation	50th Percentile Mortality					
	A-CC2 Percentage			HC-CC2 Percentage		
	010	20	0	10	20	
7	100 (100,100)	25 * (0,25)	0 * (0,0)	100 (75,100)	0 * (0,19)	0 * (0,0)
14	100 (100,100)	0 * (0,25)	0 * (0,0)	100 (75,100)	0 * (0,19)	0 * (0,0)
28	100 (100,100)	25 * (0,50)	0 * (0,25)	100 (100,100)	25* (0,25)	25 * (0,25)
90	100 (100,100)	25 * (0,25)	0 * (0,0)	100 (100,100)	0 * (0,25)	0 * (0,25)
180	100 (100,100)	75 * (75,75)	25 * (25,50)	100 (100,100)	0 * (0,0)	0 * (0,0)
365	100 (100,100)	25 * (0,25)	25 * (31,50)	100 (56,100)	0 * (0,0)	0 * (0,0)
	$X^2 = 224.6$; $df = 17$; $P = < 0.001$			$X^2 = 216.2$; $df = 17$; $P = < 0.001$		

Note. Dose of undiluted SM was 232 mg/kg (29 fold LD_{50}) applied dermally.

Dose of agents for decontamination was 200 mg smeared on the SM applied area.

Four mice were used for each day of A-CC2 and HC-CC2 percentages. 50th percentile is the percent of mice died 7 days after SM exposure. Figures in parentheses are 25th and 75th percentiles).

*Statistically significant compared to respective 0% group by Friedman's repeated measures ANOVA followed by Dunnett's method.

DISCUSSION

The mortality following SM depends upon the dose applied. Mice applied with higher doses of SM die earlier and those applied with lower doses die later. Therefore, the day of mortality will give a measure of the efficacy of the decontaminants used. Usually the animals die within 14 days after application of SM and mortality is observed rarely beyond this period[16]. Hence the mortality of the animals was observed for 14 days only. In the present study 5 µl of SM was applied, which was 29 fold LD_{50} in mice. This dose induced death within 1 week. CC2 decontaminates SM by chlorination and subsequent oxidation. In the present study 40 mg of CC2 in various hydrophilic and lipophilic formulations was used as a decontaminant (Table 2). If one molecule of CC2 neutralizesone molecule of SM (molecular weight of CC2 is 488 and that of SM is 158), stoichiometrically

TABLE 5

Decontamination Efficacy of Various Percentages of A-CC2 and HC-CC2 Formulations Against *Sulphur Mustard (SM) in Male Rats*

Days After Preparation	50th Percentile Mortality					
	A-CC2 Percentage			HC-CC2 Percentage		
	010	20	0	10	20	
7	100 (19,100)	100 (6,100)	0 (0,0)	100 (25,100)	25 (0,25)	0 (0,0)
14	100 (25,100)	50 (0,75)	0 (0,0)	100 (25,100)	25 (0,25)	0 (0,0)
28	100 (25,100)	25 (6,94)	0 (0,0)	100 (62,100)	50 (0,69)	0 (0,19)
90	100 (25,100)	50 (0,75)	0 (0,25)	100 (25,100)	0 (0,0)	25 (0,25)
180	100 (63,100)	100 (6,100)	50 (6,50)	100 (12,100)	75 (37,75)	25 (6,25)
365	100 (25,100)	100 (12,100)	50 (12,50)	100 (25,100)	100 (50,100)	0 (0,0)
	$X^2 = 170.7$, $df = 17$; $P = < 0.001$			$X^2 = 179.7$; $df = 17$; $P = < 0.001$		

Note. Dose of undiluted SM was 28.9 mg/kg (12 fold LD_{50}) applied dermally.

Dose of agents for decontamination was 200 mg smeared on the SM applied area.

Four rats were used for each day of A-CC2 and HC-CC2 percentages. 50th percentile is the percent of rats died 7 days after SM exposure. Figures in parentheses are 25th and 75th percentiles).

* Statistically significant compared to respective 0% group by Friedman's repeated measures ANOVA followed by Dunnett's method.

this should neutralize about 10 µl of SM. SM is a lipophilic chemical and gets absorbed by the skin very quickly. For an effective decontamination it has to be used within 2 min of exposure[18,21].

Chlorinating agents are strong reactants of sulphur mustard. Chloramide, known as S-330 has been reported to be a good dermal decontaminant of sulphur mustard[8,22]. The author's earlier study showed that CC2 is a good decontaminant when it is combined with Fuller's earth[17,18]. The present study was aimed in identifying a better formulation for CC2 in liquid medium for easy use. CC2 is insoluble in water and soluble only in organic solvents. Since petroleum jelly, povidone iodine ointment and calamine lotion were recommended for sulphur mustard induced wound, they were used for CC2 formulations[23]. Honey, turmeric and neem oil were selected because of their antiseptic properties. Peanut oil and silicone oil were also used for the preparation of lipophilic formulations.

Active chlorine assay is generally used for checking the stability of chlorinating compounds[24],

TABLE 6

LD_{50} Values of CC2

Species	Sex	Route	LD_{50} (g/kg)	Fiducial Limits
Mouse	Male	Oral	> 5.0	-
Mouse	Female	Oral	> 5.0	-
Mouse	Male	Intraperitoneal	0.673	370 - 1224
Mouse	Female	Intraperitoneal	0.800	382 - 1675
Mouse	Female	Dermal	> 5.0	
Rat	Male	Oral	> 5.0	-
Rat	Female	Oral	> 5.0	-
Rat	Male	Intraperitoneal	0.951	643 - 1408
Rat	Female	Intraperitoneal	1.345	909 - 1991
Rat	Male	Dermal	> 5.0	

and hence in the present study the stability of CC2 formulations was ascertained by active chlorine assay. CC2 reacts with peanut oil and neem oil as they have unsaturated oils. With povidone iodine and Fuller's earth also CC2 is unstable. Good stability was achieved with petroleum jelly, honey, polyvinyl pyrrolidone, calamine lotion, acacia and hydroxypropyl cellulose. In the present study it was observed that the preparations in honey, polyvinyl pyrrolidone, turmeric and calamine lotion dried quickly and there was difficulty in their application for decontamination. Though CC2 was stable in petroleum jelly and silicone oil, they did not protect the mice against SM, showing that the lipophilic preparations were unsuitable. The probable reason may be that the lipophilic preparations are facilitating SM absorption, as SM is highly lipophilic. But, the hydrophilic formulations particularly acacia and hydroxypropyl cellulose gave very good protection. CC2 was stable in presence of water unlike the other active chlorine containing dermal formulation S-330, where 5% to 15% water only was tolerable[24]. CC2 reacts instantaneously with SM in hydrophilic medium and the major product formed is SM sulphoxide with very little SM sulphone[25].

Further study was carried out with two different percentages of CC2, 10% and 20% in acacia and hydroxypropyl cellulose, and mice and rats were used for the efficacy study. CC2 was stable in both 10% and 20% formulations based on the active chlorine assay. Since the rat was more sensitive to SM (LD_{50} 2.4 mg/kg) a lower dose of 12 LD_{50} was used (5 µl of SM for an average weight of 220 g). In the present study it was observed that the formulation in hydroxypropyl cellulose was more fluid in nature and easy to use. With gentle shake the contents were mixed, unlike the acacia preparation which required vigourous shaking. The protection by 20% CC2 was better than 10%.

In smaller animals like mice and rats, the systemic toxicity of SM is more prominent. The LD_{50} in mice is 8.1 mg/kg and in rats is 2.4 mg/kg. All the undecontaminated animals died within 1 week and hence no skin lesions were visible. The prominent effect was reduction in body weight. The decontaminated and survived animals showed apparent skin lesions after 14 days of SM application. Since the dose applied was 29 LD_{50} in mice and 12 LD_{50} in rats, and decontamination was carried out after 2 min, skin lesions were observed. The skin lesion was more prominent in mice than in rats, and it took about a month to heal completely.

The oral and dermal LD_{50} of CC2 was above 5.0 g/kg, showing that it is a safe chemical. It is concluded that 20% CC2 in hydroxypropyl cellulose is a better decontaminant for SM

with respect to stability, efficacy and ease of decontamination.

TABLE 7

Body Weight Changes Following Oral Administration of CC2

Dose (mg/kg)	Day post Administration	Mouse		Rat	
		Male	Female	Male	Female
0	0	28.2 ± 0.45	27.8 ± 0.72	220.5 ± 6.7	186.5 ± 8.9
	1	29.3 ± 0.66	27.4 ± 0.67	224.5 ± 7.6	187.8 ± 8.6
	7	30.3 ± 0.71	28.0 ± 0.79	243.8 ± 6.7	192.0 ± 10.4
	14	32.8 ± 0.92	28.2 ± 1.01	267.3 ± 9.9	201.3 ± 12.5
1600	0	28.5 ± 0.61	27.8 ± 0.68	225.5 ± 6.3	171.3 ± 5.7
	1	28.2 ± 0.60	28.0 ± 0.52	227.0 ± 7.0	174.3 ± 4.8
	7	30.0 ± 0.63	28.1 ± 0.47	241.0 ± 7.2	171.8 ± 5.6
	14	31.7 ± 0.33	29.8 ± 0.47	260.8 ± 9.4	176.3 ± 6.3
3200	0	26.7 ± 0.25	28.8 ± 0.61	231.8 ± 9.0	192.3 ± 6.4
	1	26.8 ± 0.72	28.2 ± 0.79	235.8 ± 9.0	193.0 ± 6.5
	7	27.8 ± 1.12	29.3 ± 1.20	247.5 ± 11.9	197.3 ± 9.1
	14	31.1 ± 0.69	30.8 ± 1.29	267.3 ± 18.4	210.5 ± 8.3
6400	0	29.4 ± 1.20	27.8 ± 0.90	240.0 ± 3.4	183.5 ± 3.7
	1	26.6 ± 0.88	26.2 ± 1.01	244.8 ± 2.4	190.3 ± 3.3
	7	26.5 ± 2.49	27.3 ± 1.01	255.8 ± 4.1	198.3 ± 6.2
	14	29.0 ± 2.70	28.5 ± 0.81	265.0 ± 6.5	207.0 ± 10.4
		$F = 2.2$	$F = 1.59$	$F = 3.37$	$F = 2.14$
		$P < 0.01$	NS	$P < 0.001$	$P < 0.01$

Note. $\bar{x} \pm s$ of 4 animals. None of the groups is statistically significant from 0 mg/kg group (acacia solution only) for the same day of post administration.

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