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Original Research

Alleviating Impact of Taurine on Renal Lipid Peroxidation and Oxidative Stress in Lambda-Cyhalothrin Exposed Rat

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ABSTRACT

Background
Lambda-cyhalothrin (LCT) is an isomeric form of the two biologically active diastereoisomeric pairs of cyhalothrin, containing an alpha-cyano group. Taurine or 2-aminoethane sulfonic acid is a sulfur-containing α-amino acid that is the most abundant free amino acid in most mammal tissue.

Aim and Objectives
The present study was focused to investigate lambda-cyhalothrin induced nephrotoxicity and renal oxidative stress as well as to evaluate the alleviating role of taurine in this condition.

Methods
Lambda-cyhalothrin was administered orally at two dose levels (10.83 and 15.17 mg/kg body weight) alone or in combination after pre-treatment of taurine (50 mg/kg body weight) for consecutive 14 days.

Results
Renal toxicity was measured by a significant decrease in renal index, reduction in kidney protein and an increase in serum protein in lambda-cyhalothrin intoxicated rats. At the same time, lambda-cyhalothrin induced a significant renal oxidative stress demonstrated by elevated renal malondialdehyde content and oxidized glutathione level accompanied by a reduction in reduced glutathione and antioxidant enzymes in rats. Lambda-cyhalothrin induced renal toxicity and oxidative stress in the rat was significantly ameliorated due to the administration of taurine as an antidote.

Conclusion
All of these findings of the present study strongly suggest the protective role of taurine in the pathophysiology of lambda-cyhalothrin-induced renal toxicity and oxidative stress.

Keywords
Lambda-cyhalothrin; Taurine; Renal index; Renal toxicity; Oxidative stress.

INTRODUCTION

Now-a-days the use of pesticides in agriculture has been increasing continuously. The harmful effects of many pesticides, such as organophosphates, organochlorine and carbamates, have led to use of pyrethroids as alternatives. Pyrethroids analogs of naturally occurring pyrethrins is widely used in agriculture in many countries because pyrethroids are highly effective, low toxic to non-target organisms and have easy biodegradability.[1] Lambda-cyhalothrin (LCT) is a potent, synthetic, type II pyrethroid pesticide and is worldwide used to control a different variety of insect pests in agricultural and domestic fields and public health sectors.[2-4] It is reported that lambda-cyhalothrin is moderately toxic[5,6] for mammals and highly toxic for fish, bees and aquatic invertebrates at low concentrations.[7,8] Through the use of agricultural foodstuff, pesticide residues have the ability to affect directly on human health.[9] People those are living in proximity to farms and exposed heavily to the home application of pesticides or eat foods rich in pesticide residues, are highly vulnerable to pesticides intoxication in addition to the workers of pesticides manufacturers, agriculture workers and their families.[10] It is well documented that the accidental poisonings and death of humans occurred by the

use of pesticides especially in developing countries.[11]

Many pesticides generate their toxicity through the induction of oxidative stress.[12] The free radicals generate oxidative damage to all biomolecules and initiate a chain reaction that leads to damage in physiological systems and accumulating free radicals over a period of time cause degenerative diseases.[13] Several research studies reported the induction of oxidative stress by synthetic pyrethroids such as fenvalerate and cypermethrin.[14-16]

Taurine possesses antioxidant and membrane-stabilizing properties. Several studies reported that taurine exhibited protective activity against renal toxicity by its antioxidative role.[17] On the other study, it was reported that taurine supplementation became resistant to kidney damage and also proteinuria caused by either streptozotocin-induced type 1 diabetes or aminonucleoside-induced glomerulopathy.[18] In a related study, chronic taurine treatment prevented aging-related up regulation of transforming growth factor beta (TGF-β1), collagen types I and IV and fibronectin messenger ribonucleic acid (mRNAs), proteins involved in the development of renal fibrosis in aging rat.[19] Renal function, especially the oxidative status of the renal system may be altered due to the exposure of pyrethroids. The present study was conducted to evaluate the toxic effect of orally administered lambda-cyhalothrin on renal lipid peroxidation and antioxidant status in male Wistar rat and to find out the ameliorative potential of taurine in this toxic condition.

MATERIALS AND METHODS

Chemicals and Reagents

Lambda-cyhalothrin 5% emulsifiable concentrate (EC) was procured from RPC Agro Industries, Kolkata. Taurine, 1, 2dichloro-4-nitrobenzene (CDNB) were purchased from Sigma-Aldrich. Thiobarbituric acid, 5, 5' Dithiobis-2-nitrobenzoic acid (DTNB), ethylene di tetraacetic acid (EDTA), and hydrogen peroxide were all purchased from Sigma Chemical, USA. All other chemicals used were of the finest analytical grade.

Animal Care and Treatment

In this study, 36 mature male albino rats (Wistar) weighing 130±15 g were acclimatized for 1 week before the start of the treatments at a suitable temperature of 25 °C±2 °C with 12 hours light-dark cycle. Animals were provided with accessible dry food pellets and water sufficiently. Rats were randomly divided into six groups, and each group contains six animals. Institutional Animal Ethical Committee approved the experimental protocol. The experimental six groups were designed as:

Group-I: DW-Control (Distilled Water, 2 ml/kg body weight)
Group-II: TAU-Control (TAU, 50 mg/kg body wt.)
Group-III: LCT-Low (LCT, 10.83 mg/kg body wt.)
Group-IV: TAU+LCT-Low (TAU, 50 mg/kg body wt.+LCT, 10.83 mg/kg body wt.)
Group –V: LCT-High (LCT, 15.17 mg/kg body wt.)
Group-VI: TAU+LCT-High (TAU, 50 mg/kg body wt.+LCT, 15.17 mg/kg body wt.)

Two respective doses 10.83($1/7^{th}$ LD50 dose) and 15.17($1/5^{th}$ LD50 dose) mg/kg body wt. of LCT were applied.[20] After one hour of the treatment of taurine (50 mg/kg body wt.), lambda-cyhalothrin was administered at two dose levels (10.83 mg/kg body wt, and 15.17 mg/kg body wt.) for consecutive 14 days. Animal's weight was taken daily and the dose was adjusted accordingly to weight.

Sample Collection

The total body weight of each animal was recorded at the end of the experimental period. All rats were sacrificed by rapid decapitation after 24 hours of the last dose. Then weights of the kidney tissues were recorded and stored properly for the determination of oxidative stress biomarkers.

Estimation of Renal Index

Renal index was measured by using the following formula-

$$\text{Renal index} = \frac{\text{Kidneys weight (g)}}{\text{Body weight (g)}} \times 100$$

Estimation of Serum and Tissue Protein

Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1 mg/ ml) and water. From these different dilutions, protein reagents (98:1:1) consisting of sodium carbonate (Na_2CO_3) in 0.1 N sodium hydroxide (NaOH), sodium potassium tartrate in distilled water, copper sulphate (Cu_2SO_4) in distilled water were added to different test tubes and 10 µl of serum or tissue homogenate and 500 µl of normal saline (0.9 g%) were also added. The solutions were mixed well. Then 500 µl of Folin-Ciocalteau solution was added to each tube and incubated at 37 °C for 30 min. The standards were prepared similarly. The optical density was measured at 660 nm.[21] The absorbance was plotted against protein concentration to get a standard calibration curve.

Estimation of Oxidative Stress Parameters

Malondialdehyde (MDA): MDA of kidney tissue homogenate was assayed by using the method of Ohkawa et al.[22] One ml of homogenate (20 mg/ml phosphate buffer) was mixed with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of acetate buffer (20% pH 3.5), and 1.5 ml of aqueous solution of thiobarbituric acid (0.8%). Red pigment was produced after heating of that mixture at 95 °C for 60 min. Then it was extracted with 5 ml of n-butanol-pyridine mixture (15: 1) and centrifuged at 5000 rpm for 10 min at room temperature. The absorbance of the supernatant was measured at 535 nm.

Reduced glutathione (GSH): The reduced glutathione in kidney tissue homogenate was measured according to the method of Griffith.[23] The assay mixture contained 200 µl of kidney tissue homogenate and 100 µl of sulfosalicylic acid (4 gm %). The mixture was centrifuged for 10 min at 3000 rpm. Then 1.8 ml of DTNB (4 mg %) was added with the supernatant and was shaken well. Reading was taken at 412-420 nm.

Oxidized glutathione (GSSG): Oxidized glutathione of kidney tissue homogenate was measured by using the method of Griffith.[24] At first, 100 µl of kidney tissue homogenate was mixed with 2 µl of 2-vinyl pyridine and was incubated for 1h at 37 °C. Then 250 µl of sulfosalicylic acid (4 gm %) was added with it and was kept in room temperature for 30 min. It is centrifuged at 2000 rpm for 10 min. Then 200 µl of the supernatant was added with 2 ml of DTNB (4 mg %) and the reading was taken at 412 nm within 1min.

Catalase (CAT): CAT content was measured according to the method of Aebi.[25] The reaction mixture consisted of 0.5 ml of H_2O_2, 2.5ml of double distilled water and 40 µl of kidney tissue homogenate prepared in 0.05 M trisHCl and was taken in a cuvette. After mixing, six readings were noted at 240 nm in 30 sec interval.

Glutathione peroxidase (GPx): Glutathione peroxidase was assayed according to Rotruck et al.[26] At first, homogenates (0.2 ml) were mixed with 0.1 ml of 2.5 mM hydrogen peroxide (H_2O_2), 0.2 ml of 0.4 M sodium phosphate buffer, 0.1 ml of 10 mM sodium azide and 0.2 ml of 4 mM reduced glutathione and was incubated for 5 min at 37 °C. After that 0.4 ml of 10% trichloroacetic acid (TCA) was added to that mixture to stop the reaction and centrifuged at 3200 rpm for 20 min. Then 3 ml of disodium hydrogen phosphate (Na_2HPO_4) and 1 ml of 5, 5'-dithiobisnitrobenzoic acid (DTNB) were added to 0.5 ml of supernatant.

Statistical Analysis

All data were analyzed by One-Way analysis of variance (ANOVA) followed by two-tail *t*-test using the Origin 6.0 Scientific Data Analysis. The results were expressed as the Mean±Standard Error of Mean (SEM). The difference between group means was considered significant when $p<0.05$.

RESULTS

Effects on Renal Index

Table 1. *Effect of Taurine on Renal Index in Lambda-Cyhalothrin Exposed Male Albino Rat*

Experimental Groups	Renal Index
Group-I: DW-Control (Distilled Water, 2 ml/kg body wt.)	0.788±0.029
Group-II: TAU Control (TAU, 50 mg/kg body wt.)	0.799±0.025
Group-III: LCT-Low(LCT, 10.83 mg/kgbody wt.)	0.677±0.012a**
Group-IV: TAU+LCT-Low(TAU, 50mg/kg body wt.+LCT,10.83 mg/kg body wt.)	0.758±0.018b**
Group-V: LCT-High(LCT, 15.17 mg/kg body wt.)	0.569±0.023a***
Group-VI: TAU+LCT-High (TAU, 50mg/kg body wt. +LCT, 15.17 mg/kg body wt.)	0.729±0.035c**

Results are expressed as Mean±SEM (N=6). The analysis is done by ANOVA followed by multiple comparison two-tail *t*-tests. Superscript a: Group-I *versus* all other groups; Superscript b: Group-III *versus* Group-IV; Superscript c: Group-V *versus* Group-VI. Asterisks represent the different level of significance (** indicates $p<0.01$, *** indicates $p<0.001$).

The renal index of LCT exposed rats was decreased significantly ($p<0.001$) in a dose-dependent manner compared to a control group (Table 1). Taurine increased the renal index of LCT induced rats significantly.

Effects on Kidney and Serum Protein Content

In the present study, lambda-cyhalothrin caused a reduction in total kidney protein level compared to control rats in a dose-dependent manner. At the same time, LCT increased the serum protein level in LCT-exposed rat. Taurine restored back the respective protein levels towards normal in both of the cases (Figures 1 and 2).

Figure 1. *The Effect of Taurine on Kidney Tissue Protein in Lambda-cyhalothrin Exposed Male Albino Rat. Results are Expressed as Mean±SEM (N=6). Analysis is Done by ANOVA Followed by Multiple Comparison Two-Tail t-tests. Superscript a, Group-I versus all Other Groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks Represent the Different Level of Significance (* indicates p<0.05, **indicates p<0.01)*

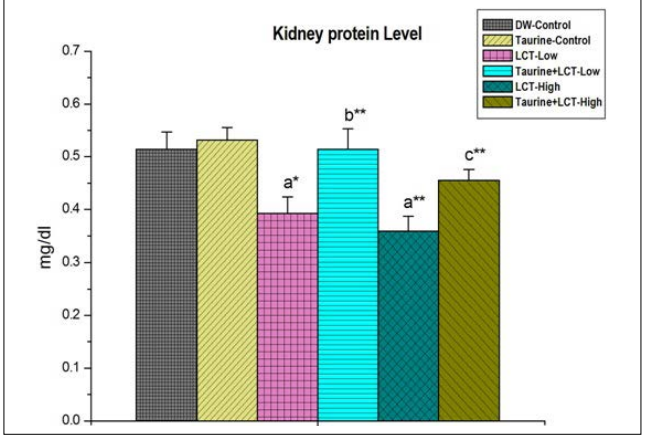
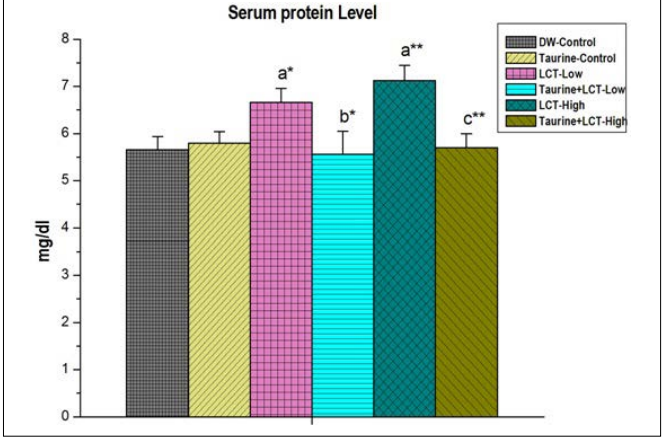


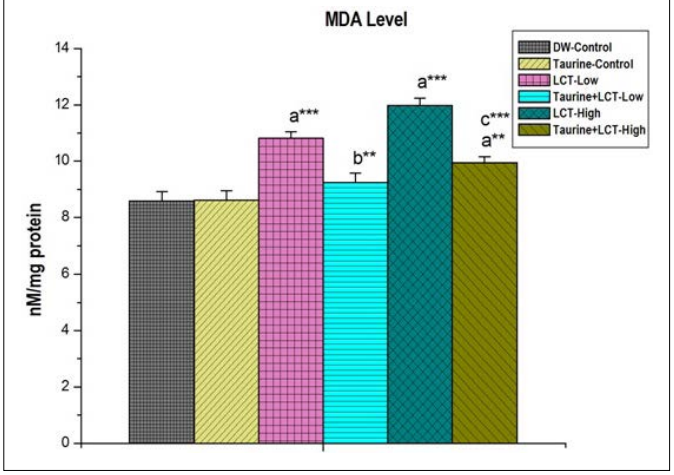
Figure 2. *The Effect of Taurine on Serum Protein in Lambda-cyhalothrin Induced Male Albino Rat. Results are Expressed as Mean±SEM (N=6). Analysis is Done by ANOVA Followed by Multiple Comparison Two-Tail t-tests. Superscript a, Group-I versus all Other Groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks Represent the Different Level of Significance (* indicates p<0.05, **indicates p<0.01)*



Effects on Enzymatic Parameters for Lipid Peroxidation

The effect of taurine on kidney malondialdehyde (MDA) level of lambda-cyhalothrin exposed male albino rat is shown in Figure 3. In LCT treated group, MDA content increased significantly (p<0.001) compared to the control group in a dose-dependent manner where treatment of taurine decreased the LCT toxicity and restored the normal level of the MDA to a great extent.

Figure 3. *The Effect of Taurine on Kidney Malon-di-Aldehyde (MDA) Level in Lambda-cyhalothrin Exposed Male Albno Rat. Results are Expressed as Mean±SEM (N=6). SuperScript a, Group-I versus all Other Groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks Represent the Different Level of Significance (**indicates p<0.01,*** indicates p<0.001)*



Kidney GSH level was decreased in LCT low and high dose treated animal groups significantly (p<0.001) but pre-treatment of taurine causes significant (p<0.001) elevation in GSH level in LCT intoxicated animals (Figure 4).

Figure 4. *The Effect of Taurine on Kidney GSH Level in Lambda-cyhalothrin Exposed Male Albino Rat. Results are Expressed as Mean±SEM (N=6). Analysis is Done by ANOVA Followed by Multiple Comparison Two-Tail t-tests. Superscript a, Group-I versus all Other Groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks Represent the Different Level of Significance (* indicates p<0.05, *** indicates p<0.001))*

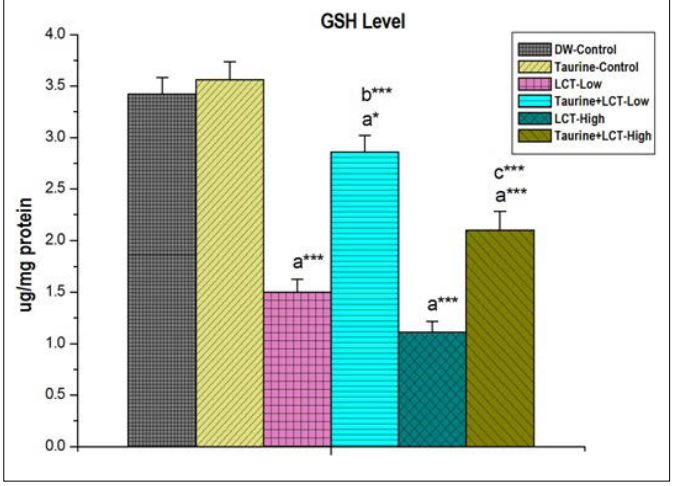
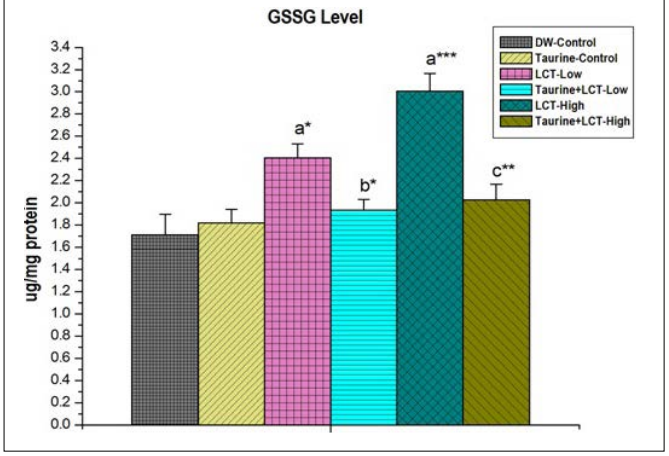


Figure 5 shown that the kidney GSSG level in LCT low and high dose treated rats were increased significantly (p<0.05 and p<0.01) compared to control rats. GSSG level was decreased by taurine pre-treatment in LCT exposed rats.

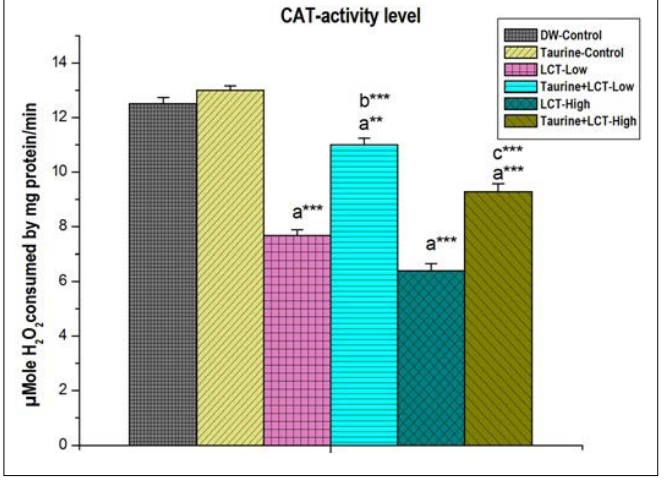
Figure 5. *The Effect of Taurine on Kidney GSSH Level in Lambda-cyhalothrin Exposed Male Albino Rat. Results are Expressed as Mean±SEM (N=6). Analysis is Done by ANOVA Followed by Multiple Comparison Two-Tail t-tests. Superscript a, Group-I versus all Other Groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks Represent the Different Level of Significance (*indicates p<0.05,**indicates p<0.01,***indicates p<0.001)*



Effects on Antioxidant Enzymes

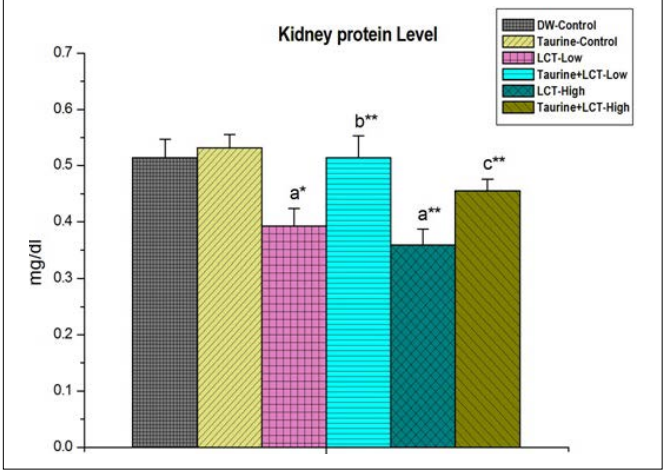
As presented in Figure 6, the activities of CAT in the LCT treated low and high dose groups were significantly (p<0.001) decreased compared to the control group. However, the activity of CAT was significantly increased by taurine pre-treatment in low (p<0.01) and (p<0.05) high dose group animals.

Figure 6. *The Effect of Taurine on Kidney Catalase (CAT) in Lambda-cyhalothrin Exposed Male Albino Rat. Results are Expressed as Mean±SEM (N=6). Analysis is Done by ANOVA Followed by Multiple Comparison Two-Tail t-tests. Superscript a, Group-I versus all Other Groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks Represent the Different Level of Significance (**indicates p<0.01, *** indicates p<0.001)*



Activities of glutathione peroxidase (GPx) in kidney of LCT treated low and high dose animals were significantly (p<0.05) and (p<0.001) decreased than the control group rats. Taurine treatment significantly increased GPx levels, in low dose (p<0.05) and high dose (p<0.01) animals (Figure 7).

Figure 7. *The Effect of Taurine on Kidney Glutathione Peroxidase (GPx) in Lambda-cyhalothrin Exposed Male Albino Rat. Results are Expressed as Mean±SEM (N=6). Analysis is Done by ANOVA Followed by Multiple Comparison Two-Tail t-tests. Superscript a, Group-I versus all Other Groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks Represent the Different Level of Significance (* indicates p<0.05,**indicates p<0.01)*



DISCUSSION

The present study was designed to evaluate the toxic effects of lambda-cyhalothrin on male albino rat kidney and its attenuation by taurine. It has been reported that in toxicological studies, body and organs weights are considered as important criteria for evaluating organ toxicity. The body weight change is considered as a sign of toxicity of any chemical substance.[27] In this study, we have evaluated the renal index as the basic marker for the renal toxicity. The renal index of lambda-cyhalothrin intoxicated rats was significantly lower than control rats but attenuation of renal index by taurine was seen and this may be due to its antioxidant activity. Ingestion of chlorpyrifos, diazinon and their mixture resulted in the reduction in relative kidney weight in male rats which is in agreement with our findings.[28] In addition; one of the most common reasons for renal tissue damage is oxidative stress which was observed in lambda-cyhalothrin exposed rats.

Proteins, the essential organic macromolecules for cellular structure and function, are expected to react first with pesticide after the entry of pesticide into body cells. Pesticides have the ability to alter very quickly the buffering system of the intracellular environment. Pesticides impair protein metabolism leading perhaps to a disarray of functional and structural status of the cell.[29] In the present study, lambda-cyhalothrin had a significant lowering effect on total serum protein levels in dose-dependent manner compared to control rats.

Toxicants can cause a defect in protein synthesis and that may lead to a decrease in tissue protein content. The exposure of toxins to living organisms may alter the hormonal balance that can results a direct or indirect decrease in tissue protein content.[30,31] The effect of different pesticides such as endosulfan,[32] organchlorins,[33] chlorpyrifos,[34] phosphorothionate,[35], imidacloprid,[36] and cypermethrin[37] poisoning on protein metabolic profiles of rats has been studied by different researchers. In current investigation exposure of lambda-cyhalothrin to albino rats resulted in a gradual decrease in the protein content of kidney tissue.

Oxygen free radical induced lipid peroxidation, which causes damage to cell membranes and consequently develops tissue injury.[38] In this study, lambda-cyhalothrin elevated renal malondialdehyde (MDA) level and reduced renal glutathione (GSH) contents as well as inhibited glutathione-s-transferase activity of the kidney tissue. The decrease in tissue GSH due to the enhancement in lipid peroxidation is considered as an antioxidant defence role of GSH. GSH, glutathione-s-transferase are used in the cell as antioxidant defence mechanism. Reduced glutathione serves as an antioxidant against free radicals and organic peroxide.[39]

LCT induced toxic manifestations may also be associated with the induction of oxidative stress through the formation of free radicals and alteration in antioxidant systems. It was observed that LCT significantly increased the level of MDA in the kidneys of rats, whereas the activity of antioxidant enzymes (CAT) was decreased.[40] Treatment with taurine caused a significant reduction in the toxic effects of this pesticide. The administration of LCT in different periods of postnatal ontogenesis was also reported to enhance oxidative stress by a significant increase in MDA level and suppressed activity of antioxidant enzymes (CAT) in brain tissue.[41] In our study, we found that administration of LCT to rats resulted in a marked dose-dependent increase in the lipid peroxidation as indicated by the increase in the level of malondialdehyde (MDA) and that may be due to LCT induced increase in ROS level. GSH, one of the most important biological molecules, play a key role in the detoxification of the reactive toxic metabolites. Decline in GSH levels in the kidney after LCT treatment may be an indication of oxidative stress, whereas GSH is utilized for the detoxification of reactive toxic substances. An increased level of GSSG also reflects the oxidative stress of ovary. Normal cellular functioning depends on a balance between ROS production and antioxidant defence mechanisms present in the cell.

Antioxidant enzymes cause a primary defence that prevents oxidative damage of biological macromolecules. According to the results, the activities of CAT, a glutathione peroxidase in the kidney of LCT treated rats were significantly decreased. These results suggested that LCT has the capability to induce free radicals and oxidative damage as evidenced by alterations in various antioxidant enzymes.[42] Reduction of antioxidant enzymes levels may be due to the direct effect on the enzymes against LCT-induced ROS generation. Taurine administration reversed all these abnormalities of above mentioned renal parameters to a good extent. It diminished lipid peroxidation either by scavenging or quenching oxygen-derived free radicals, hydrogen peroxide or hypochlorous acid directly, or by binding free metal ion species like Fe^{2+} or Cu^{2+} by its sulfonic acid group. It was also suggested that by decreasing

carbonyl group production, enhanced oxidative damage[43] was reduced by taurine.[17]

CONCLUSION

The present findings demonstrated that taurine was able to reverse the pathological parameters of renal damage induced by lambda-cyhalothrin. Pre-treatment of taurine maintained the antioxidant status of kidney due to its free radical scavenging action. Taurine undoubtedly restored the renal function by blocking lambda-cyhalothrin induced renal oxidative stress. So, taurine may be considered useful against lambda-cyhalothrin induced toxicity in renal system.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Systematic Review

Poisonings in Singapore: A Poison Center Perspective

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ABSTRACT

The Drug and Poison Information Center (DPIC) in Singapore was run as a pilot project over 4 years from April 2004 to March 2008. The center provided a hotline service for toxic exposure assessment and management to healthcare professionals and the general public. The aim of this study was to review poisonings through the perspective of this poison center.

Method

A retrospective review of records in the DPIC call database was made covering the 4 years of its operation. Drug information and adverse effects calls were excluded from the study.

Results

There was a total of 15227 calls to the DPIC over the study period. Of these, 1817 calls (11.9%) were on acute toxic exposures involving patients. Healthcare personnel working in public restructured hospitals were the most frequent users (71.4%) of the service with the majority of these calls originating from the emergency departments (86%). Public inquiries accounted for 16.6% of the call volume. The cohort of poisoning cases showed a bimodal distribution of age groups with peaks in the less than 5 age group and the 20 to 40 year age group. The racial distribution followed local population demographics but with almost equal gender representation (50.3%males). Most exposures were accidental (67.4%) and occurred at home (69%). The number of agents involved in each exposure ranged from one (84.5%) to a maximum of 6 (<1%) agents. The common exposures involved analgesics (13.5%), antidepressants and sedatives (10.6%), industrial chemicals (5.7%) and bites and stings (8.4%). The calls were evenly distributed by month of the year with no significant seasonal variation although the daily distribution showed a peak in the late evening. The DPIC was able to complete immediate definitive advice within 15 minutes of the call in most situations (96.5%). Majority of public calls (69.2%) ended with reassurance and advice to observe for relevant symptoms. A similar disposition was observed even when the calls were from physicians.

Conclusion

In summary, poisonings were mostly accidental and affected the younger population suggesting that they are potentially preventable. Furthermore, the DPIC appears to have played a significant triaging role in toxic exposures; providing reassurance for minor poisoning cases while facilitating the appropriate referral of the more severe ones.

Keywords
Poison center; Toxic exposures; Poisoning; Overdose; Singapore; Demographics.

INTRODUCTION

The Drug and Poison Information Center (DPIC) in Singapore was run as a pilot project over 4-years from April 2004 to March 2008. The primary objective of the center was to provide a telephone consultative service to both healthcare professionals and the general public to assist with toxic exposure assessment and recommendations for optimal medical management. In addition, drug information and adverse reactions advisory services were also provided. This service was provided free at no cost to the end user. The aim of this study was to analyze the demographics of poison exposures from the perspective of this pilot poison call center.

METHOD

Drug and poison information inquiries were captured and entered into a formatted database by pharmacists and poison information specialists providing caller assistance at the DPIC. A retrospective review of poisons records stored in the poison information center call database was made covering the entire period of its operations

from April 2004 to March 2008. Drug information and adverse effects calls were excluded from the study and only toxic exposure calls were analyzed. Demographic data, toxic exposure, advice provided and outcome information were analyzed.

RESULTS

There was a total of 15227 calls to the DPIC since its operation on April 04 to March 2008. Of these 13364 calls were excluded as they covered drug information and adverse drug reaction related inquiries. A further 46 calls were excluded as they were inquiries on toxins with no patient involvement. The remaining 1817 (11.9%) included in the study were on acute toxic exposures involving patients.

DPIC User Profile

Healthcare workers in public hospitals were the most frequent users (71.4%) of the DPIC service (Table 1), predominantly from the Emergency Departments (86%). Overall, physicians (78.8%) were the primary users of the service with majority originating from junior level medical staff (70%) including house officers and medical officers. Calls from members of the public mainly non-medical persons accounted for 16.6% of the call volume.

Table 1. *Origin of Poison Information Calls*

Location	Number of calls
Public Restructured hospitals	1296 (71.4%)
Private hospitals	53 (2.9%)
GP Clinics	108 (5.9%)
Other healthcare institutions*	32 (1.8%)
Other+	26 (1.4%)
Member of Public@	302 (16.6%)
Total	1817(100%)

*Includes polyclinics, private pharmacies, National Dental Centre, National Cancer Centre, National Heart Centre, National Neuroscience Institute, National Skin Centre, and Singapore National Eye Centre.
+Includes research institutions, pharmaceutical companies, government organizations.
@includes self, relatives, friends, colleagues, witnesses

Toxic Exposure Patient Demographics

The age of patients ranged from 3 months to 99 years (mean age 21.1 years) with a bimodal distribution with peaks in the under 5 age group and the 20 to 40 year age group (Table 2). The racial distribution followed local demographics with almost equal gender representation with 50.3% of the cohort being males.

Toxic Exposure Incident Information

The most common site of incidence was home (69%) and the majority was of an accidental nature (67.4%) (Table 3). The number of agents involved in each exposure ranged from one (84.5%) to a maximum of 6 (<1%) co-ingestants. Prescription medications were responsible for most exposures (46.8%) Table 4 with exposures to analgesics (13.5%), antidepressants and sedatives (10.6%), industrial chemicals (5.7%) and bites and stings (8.4%) forming the bulk of agents involved. The commonest route of exposure was oral (70.4%).

Table 2. *Age Distribution of Poisoning Cases*

Age (years)	Number of incidents (Total)
0-5	425 (37.1%)
6-10	50 (4.4%)
11-15	68 (5.9%)
16-20	98 (8.6%)
21-30	203 (17.7%)
31-40	133 (11.6%)
41-50	75 (6.6%)
51-60	38 (3.3%)
61-70	19 (1.7%)
>70	37 (3.2%)
Total	1146
Missing data	671
Total	1817

Table 3. *Toxic Exposure Incident Site*

Place	Number of incidents
Home	1072 (69%)
Workplace	116 (7.4%)
Public areas*	242 (15.6%)
Unknown	124 (8%)
Total	1554 (100%)
Missing data	263
Total	1817

*Includes places such as beach, parks, and other recreational placese.g. pubs

The distribution of calls during the DPIC pilot phase is shown in Figure 1. The calls were evenly distributed by month of the year with no significant seasonal variation except for a slight dip in the middle of the year and a more significant number in the second half of the year (Figure 2). There was also no significant daily variation by day of the week except for a notable dip in calls on Sundays. The daily distribution of calls showed a peak at 1500-hours and 2200-hours with an equitable distribution between office (0800-1700 hours over 9-hours) and after office hours (1700 hours till 0800-hours the following day) (Figure 3). A proportionally larger distribution of toxic exposure calls occurred outside working hours during the late evenings and nights as well as public holidays.

Poison Center Intervention and Outcome

It is noted that for most calls from the public (69.2%), the poison center advice was to reassure and observe the patient with no recommendation for physician visits (Table 5). There was a similar disposition even when the calls were from community phy-

Table 4. *Agents Used in Poisoning*

Agent	Number of agents (% of total exposures)
Acids/Alkaline/Corrosives	65 (3%)
Alcohol	33 (1.5%)
Analgesics (excluding paracetamol)	119 (5.5%)
Analgesics- Paracetamol	175 (8%)
Antidepressants	102 (4.7%)
Antihistamines	112 (5.1%)
Antimicrobials	2 (0.1%)
Antipsychotics	46 (2.1%)
Asthma medications	26 (1.2%)
Cardiac medications	51 (2.3%)
GI medicines	17 (0.8%)
Sedatives	129 (5.9%)
Other Western medicines	241 (11.1%)
Traditional Medicine	33 (1.5%)
Bites and Stings	183 (8.4%)
Pesticides	87 (4%)
Household Cleaning Products	128 (5.9%)
Cosmetics	29 (1.3%)
Food products/substances	24 (1.1%)
Illicit Drugs	13 (0.6%)
Industrial Chemicals	124 (5.7%)
Smoke Inhalation	20 (0.9%)
Vitamins/mineral supplements/ OTC* products	41 (1.9%)
Others+	361 (16.6%)
Unknown	18 (0.8%)
Total	2179 (100%)

Note that some exposures may involve more than 1 agent.
+includes silica gel, etc.
*OTC (over the counter)
281 (15.5%) incidents involved >1 agent

Figure 1: *Distribution of Poison Call Volume 2004-2008*

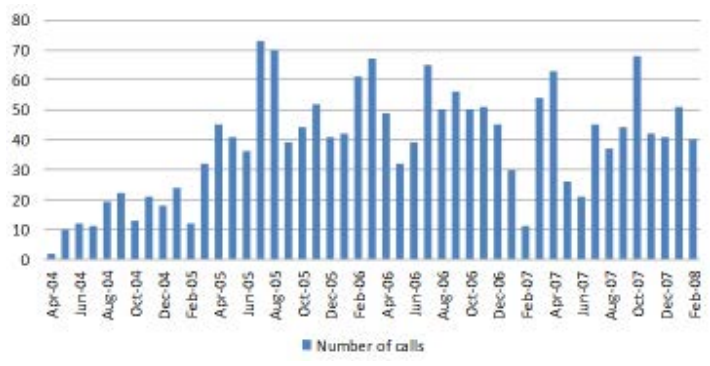

Figure 2: *Distribution of Calls by Month*

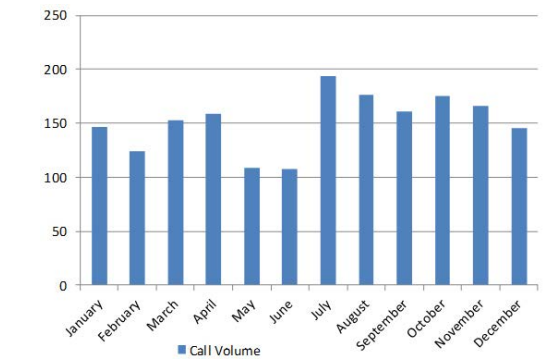

Figure 3: *Poison Call Volume by Time of Day*

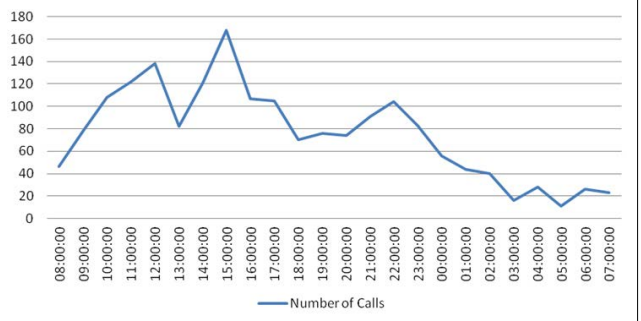

Table 5. *Poison Center Intervention - Advice to Caller*

Poison Center Intervention	Number of Calls (%)	Total Number of Calls (% of All Calls to DPIC)
Public Calls		302 (16.6%)
Advice to go to hospital	70 (23.2%)	
Advice to see a GP	23 (7.6%)	
Advice to be observed at home	209 (69.2%)	
Community Healthcare Calls		166 (9.1%)
Advice to go to hospital	38 (22.9%)	
Advice to be observed at home	128 (77.1%)	
Emergency Department (ED) Calls		1115 (61.4%)
Advice to admit	321 (28.8%)	
Advice to observe and discharge	794 (71.2%)	
Calls from the Ward		234 (12.9%)
All Calls to Drug and Poison Information Center (DPIC)		1817 (100%)

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sicians (77.1%) and emergency department physicians (71.2%). This potentially demonstrates the triaging function of a poison center reducing unnecessary healthcare visits and saving time and healthcare cost by empowering the public and community physicians while improving the quality of care of poisoning cases with appropriate management and referral advice. This advisory service would serve as an even more critical resource in a chemical disaster involving exposure of a large population.

The DPIC has been able to provide immediate definitive advice within 15 minutes of the call for most situations (96.5%) and 99.5% of all calls were resolved within one hour (Table 6). The remaining smaller proportion of cases took up to 8 hours to be resolved due to complexities of the cases involved since the detailed search for information took up most of the time.

Table 6. *Response Time for Poison Information*

Time taken	Number of calls (total)
Immediate	1353 (74.5%)
<15mins	400 (22%)
15–60 mins	54 (3%)
1-8 hrs	6 (0.3%)
8-24 hrs	3 (0.2%)
>24 hrs	1 (0.1%)
Total	1817

DISCUSSION

The number of toxic exposures presented in this study was small, most likely due to the limited publicity of this service. Based on the official statistics, there was a total of 4990 cases of individuals being admitted to Singapore hospitals following an episode of poisoning between 2004 and 2006.[1] Although the overall incidence of poisoning fell slightly during this period, it remained (coupled with accidents and violence) the most common cause of hospital admissions.

Being in the frontline of emergency services, emergency department (ED) doctors routinely manage toxic exposures and have significant clinical experience in managing poisonings. Contrary to expectation, these doctors working in the ED setting were noted to use the service more frequently (86%). The reason for this may be multifactorial, including varying experience and comfort level amongst ED physicians on managing toxic exposures to a myriad of agents with limited information resources and staffing issues comprising a significant proportion of junior doctors rotating through the ED. The latter is suggested as junior level doctors were noted to use the service more frequently accounting for 70% of all physician users.

There are several notable differences comparing toxic exposures from the ED[2] and DPIC perspectives. The toxic exposures from the ED perspective showed that the mean age of poisoning was 31.8-years with predominance of males 63.3% compared to 21.1-years and 50.3% respectively from the DPIC perspective. The proportion of non-accidental poisonings was also larger in the ED cohort (60%) compared to the DPIC (32.6%). There is insufficient data in the study to determine the reason for the difference and would be an area for future research.

In both studies the commonest site of exposure was the home and the common agents were analgesics, sedatives, bites and industrial chemicals but alcohol related exposures were more common in the ED setting. A study by Wai et al determined the incidence of attempted suicide amongst young people treated in a local teaching hospital between 1991 and 1995.[3] Females were the predominant gender committing self-harm by poisoning and the most common medication used was analgesics with paracetamol-based products being the most common. Similar results were obtained in another study performed in Northern Malaysia, a neighbouring country which share close cultural and economic ties with Singapore.[4] In Hong Kong, a regional Asian country with a poison information centre established in 2005, 8.4% of poisonings involved the use of paracetamol, representing one of the most common agents used in poisoning similar to our study.[5] Based on the 2017 American Association of Poison Control Centres (AAPCT) 2017 National Poison Data System (NPDS) annual report,[6] analgesics (11.08%) which include paracetamol is amongst the top five toxin classes involved in human toxic exposures. The ready availability of paracetamol as an over the counter drug may not completely address the reason for this coincidence.

The DPIC appears to have played a significant role in toxic exposures management; advising and reassuring minor poisoning cases while facilitating the appropriate referral of the more severe cases to the hospital ED for further management. This potentially demonstrates the triaging function of a poison center reducing unnecessary healthcare visits and saving time and healthcare cost.

In addition, it is noted that the proportion of patients admitted as advised by the DPIC (28.8%) was smaller compared to the previous study on toxic exposures presenting to the ED from 2001 to 2003, when poisoning admissions were notably higher (36.1%) when patients were managed in the ED without access to a DPIC service. There appears to be more effective utilization of limited hospital bed resources with the use of the DPIC service.

CONCLUSION

This study provides a historical baseline for toxic exposure statistics of the past which will be useful for analyzing current and future trends in poisoning.

It is notable that young people tend to be vulnerable to toxic exposures and the majority are accidental and hence potentially preventable. The role of poison prevention education for parents with young children and poison proofing homes may be potentially beneficial in reducing the number of accidental poisonings in the home.

The cost effectiveness[7] and user friendliness[8] of the DPIC were noted in prior studies and with the current evidence of good clinical outcomes through DPIC services demonstrates the value of the DPIC as a community resource in managing poisonings.

LIMITATIONS

There was limited publicity on the services of the DPIC and this may have contributed to the low numbers of calls that were handled. Data capture was incomplete in many variables and this limited the validity of conclusions drawn.

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CONFLICTS OF INTEREST

There is no conflict of interest.

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Brief Research Report

A Cycle of Altered Proteasome and Reactive Oxygen Species Production in Renal Proximal Tubular Cells

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ABSTRACT

Aims
An intricate relationship exists between the mitochondrial function and proteasome activity. Our recent report showed in a rat model of renal transplantation that mitochondrial dysfunction precedes compromised proteasome function and this results in a vicious cycle of mitochondrial injury and proteasome dysfunction. In this study, we studied whether reactive oxygen species (ROS) has a role in proteasome alteration in renal cells and *vice versa*.

Methods
We used the genomic and pharmacologic approach on rat normal kidney proximal tubular (NRK) cell lines. First, we knocked down β5 or Rpt6 subunit of the proteasome using small interfering RNA (siRNA) in NRK cells. We also treated NRK cells with Bortezomib, a proteasome inhibitor, and peroxynitrite (a potent ROS).

Results
Studies with RNA interference showed increased mitochondrial ROS following knockdown of β5 or Rpt6 subunit in NRK cells. Similarly, pharmacological inhibition of the proteasome in NRK cells using Bortezomib also showed an increase of mitochondrial ROS in a dose-dependent manner. Next, exposing NRK cells to different concentrations of peroxynitrite provided evidence that the higher levels of peroxynitrite exposure decreased the key subunits (β5 and α3) of the proteasome in NRK cells.

Conclusion
Our results suggest that proteasome inhibition/downregulation increases ROS, which then impairs proteasome subunits in renal proximal tubular cells.

Keywords
Ubiquitin-proteasome system (UPS); Reactive oxygen species (ROS); Renal proximal tubular cells.

INTRODUCTION

The proteasome is the main machinery of the ubiquitin-proteasome system (UPS), which is essential for maintaining protein quality in all eukaryotic cells.[1-3]. The proteasome is composed of a cylindrical 20S proteasome and one or two 19S regulatory particle (s), both of which participate in selectively degrading ubiquitin-tagged proteins. The 20S proteasome has 3 to 7 protease active sites (β-catalytic subunits) that hydrolyze peptide bonds in chymotrypsin (β5)-, trypsin (β2)-, or caspase (β1)-like fashion.[2] A functional proteasome plays a crucial role in degrading modified, misfolded, or damaged proteins to maintain intracellular protein homeostasis in kidneys. Therefore, any alteration to its components has the potential to disrupt protein homeostasis and could lead to pathological consequences.[4-6]

Excessive ROS generation is implicated in the pathogenesis of ischemia-reperfusion-induced renal damage.[7] Studies suggest that ROS play a complex role in modulating proteasome activity. However, the role of the proteasome pathway during renal

ischemia-reperfusion needs to be fully elucidated.[8] Interestingly, *in vitro* data show that ROS exposure to mammalian cells can inhibit the proteasome function and can alter its composition.[9,10] We recently demonstrated that mitochondrial dysfunction precedes compromised proteasome function in a rat model of renal cold storage plus transplantation, and reported the existence of a functional interdependent relationship between the proteasome activity and mitochondrial function in rat kidneys/renal cells.[11] The main goal of this study was to examine a relationship between ROS and proteasome alteration in renal proximal tubular cells. Using normal rat kidney proximal tubular cell line (NRK), here, we demonstrate that proteasome inhibition increases mitochondrial ROS and exogenous ROS treatment declines proteasome subunit level.

METHODS

Cell Culture

Normal rat kidney proximal tubular cell line cultures (NRK-52E; ATCC No. CRL-1571) a.k.a. NRK cells were used in this study. The cells were maintained in growth medium (DMEM plus 5% fetal calf serum and 1% penicillin/streptomycin) and 5% CO_2 incubator at 37 ºC as described by the American Type Culture Collection (ATCC).

Cell Treatment

NRK cells were seeded a day before small interfering RNA (siRNA) transfection or Bortezomib or peroxynitrite treatment

a) siRNA transfection: NRK cells were transiently transfected with sigenomeβ5 (PSMB5) siRNA SMART pool or Rpt6 (PSMC5) siRNA SMART pool (100 nM) (Dharmacon, USA) using siRNA transfection reagent (Invitrogen, USA) in OPTI-MEM (Invitrogen, USA) for 24 hours at 37 °C (as suggested by the manufacturer). A similar concentration of scrambled siRNA (Dharmacon, USA) was used as a control. The next day, cells were either harvested for protein extract or evaluated for ROS production (see MitoSOX™ Red fluorescence).

b) Bortezomib treatment: Bortezomib (BTZ) is a specific inhibitor of the β5 subunit of the proteasome.[12,13] NRK cells were treated with BTZ (0, 10, 20, and 50 nM for 4 hr; Selleckchem, USA) in the normal growth medium. NRK cells treated with the same concentration of DMSO (no BTZ) were used as vehicle control. After 4 hrs, cells were evaluated for ROS production (see MitoSOX™ Red fluorescence).

c) Peroxynitrite treatment: Growth medium was removed, NRK cells were washed with PBS (pre-warmed at 37 ºC), treated with peroxynitrite (30 or 300 μM; Calbiochem, USA) in warm PBS (37 ºC) for 20 minutes. After 20 minutes, the PBS was removed, and normal growth medium added to the cells and cultured for 4 hr. NRK cells treated with the same volume of degraded peroxynitrite were used as vehicle control.

Reactive Oxygen Species (MitoSOX™ Red Fluorescence) Measurement

MitoSOX™ Red reagent (Invitrogen Molecular Probes, USA) is a fluorogenic dye specifically targeted to mitochondria in live cells. Oxidation of MitoSOX™ Red reagent by superoxide produces a bright red fluorescence.NRK cells were preloaded with MitoSOX™ Red reagent (5 μM, Molecular Probes, USA) for 10 min prior to Bortezomib treatment or siRNA transfection (against β5 or Rpt6 subunit). After 4 hrs of BTZ treatment or 24 hrs of siRNA transfection, growth medium from NRK cells was replaced with warm PBS. Red fluorescence was then visualized using a Nikon Eclipse E800 microscope with a rhodamine filter using a water immersion objective (60X). All images were captured with equal exposure times. Fluorescence intensity of the captured image was evaluated using Image J software. Corrected total cell fluorescence (CTCF) was calculated as described by Martin Fitzpatrick, University of Birmingham, United Kingdom, using the following formula: CTCF=Integrated Density-(Area of selected cell X Mean fluorescence of background readings).

Renal Extract Preparation for Western Blot

Renal extracts from whole-kidney homogenates and NRK cells were prepared with radioimmunoprecipitation assay (RIPA) lysis buffer containing 1mM phenylmethylsulfonyl fluoride (PMSF), 1.2 mM Na_3VO_4, 2.5 mM NaF, and 1 mM DTT (Sigma-Aldrich, USA) and protease inhibitor cocktail (Pierce, USA). [11] After lysis, the extracts were centrifuged (16000 g for 20 min at 4 °C), and the supernatant was saved as the NRK cell extract. Protein concentrations were determined with the BCA Protein Assay kit (Pierce, USA). Renal extracts (20 μg) were separated by SDS-PAGE and transferred to a PVDF membrane. The membranes were incubated with antibodies to β5 subunit (1:1000; Abcam, #ab3330), α3 subunit (1:1000; Abcam, #ab119419), or β-actin (loading control, 1:1000; Sigma-Aldrich, #A5441). Probed membranes were washed three times, incubated with horseradish peroxidase-conjugated secondary antibodies (1:30,000; Seracare KPL), and assayed for enhanced chemiluminescence (Thermo Fisher Scientific, USA). Densitometry was performed with AlphaEase FC software (Alpha Innotech, USA).

Statistical Analysis

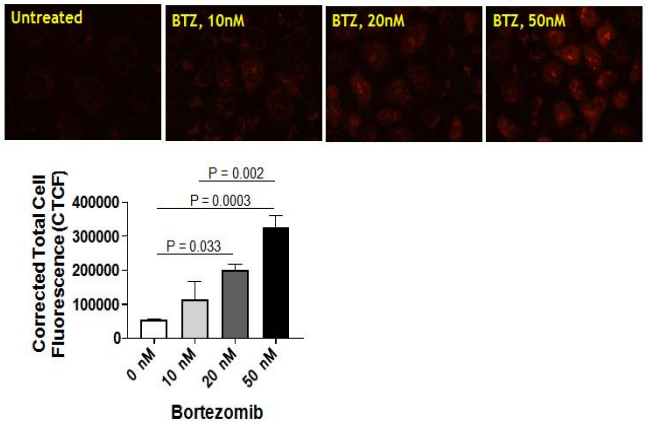
Results are presented as the mean±standard error of the mean (SEM) (GraphPad Prism software, USA). Data (n=4-6 assays) were analyzed with a one-way ANOVA and Tukey's posthoc test for multiple group comparisons, and an unpaired Student's *t*-test was used when comparing differences between the means of two groups (Control *versus* CS) at a 95% level of confidence. Differences with $p<0.05$ were considered statistically significant.

RESULTS

Bortezomib treatment increases mitochondrial ROS in NRK cells. We recently reported that Bortezomib (BTZ) treatment increas-

es mitochondrial dysfunction and alteration of key respiratory subunits in NRK cells.[11] This finding prompted us to determine whether BTZ treatment also increases the mitochondrial ROS. In this study, MitoSOX™ Red (Invitrogen Molecular Probes, USA) was used to detect mitochondrial ROS production in NRK cells. This modified cationic dihydroethidium dye is localized to the mitochondria where it is oxidized by superoxide to generate a bright red fluorescence.[14] Interestingly, the mitochondrial ROS was increased after BTZ treatment of NRK cells in a dose-dependent manner (Figure 1). The vehicle control treatment had no effect on mitochondrial superoxide generation in NRK cells (Figure 1).

Figure 1. *(a) Bortezomib Treatment Increases Mitochondrial Reactive Oxygen species in a Dose-dependent Manner. Normal Rat kidney Tubular (NRK) Cells were Preloaded with MitoSOX™ Redreagent Followed by Pharmacological Inhibition of the Proteasome using Bortezomib (BTZ; 0 -50 nM) for 4 hrs. DMSO Treated NRK Cells were Used as Bortezomib Treatment Increases Mitochondrial Reactive Oxygen Species in a Dose-dependent Manner. Normal rat Kidney Tubular (NRK) cells were Preloaded with Red reagent followed by Pharmacological Inhibition of the Proteasome using Bortezomib (BTZ; 0 -50 nM) for 4 hrs. DMSO Treated NRK Cells were used as Vehicle Control. Red Fluorescence was then Lisualized Using a Nikon Eclipse E800 Microscope with a Hodamine Filter Using a Water Immersion Objective (60X). All Images were Captured with Equal Exposure Times and the Fluorescence Intensity was Evaluated Using Image J Software by Calculating Corrected Total Cell Fluorescence (CTCF) (please refer to Methods section). Results are Representative of 6 Independent Analyses for Each Group and Thevalues are Expressed as the Mean±SEM (bars)(n=6). Differences Between Means were Compared with a One-way ANOVA for Multiple Group Comparisons (Vehicle, 10nM BTZ, 20n M BTZ, and 50 nM BTZ).*



Knockdown of proteasome subunit increases mitochondrial ROS in NRK cells. Given pharmacological inhibition of the proteasome increases mitochondrial ROS production, further studies used RNA interference to assess mitochondrial ROS production in NRK cells. We found increased mitochondrial ROS production in NRK cells transfected with β5 (a 20S proteasome subunit) or Rpt6 (a 19S proteasome subunit) siRNA (Figure 2). As anticipated, scramble siRNA did not affect the mitochondrial ROS generation (Figure 2).

Peroxynitrite treatment altered proteasome subunit levels in NRK cells. Because we found that BTZ treatment or siRNA mediated knockdown of β5 or Rpt6 subunit increases ROS, here we attempted to evaluate whether exogenous ROS exposure alters proteasome subunit levels. We solubilized NRK cells with RIPA buffer to extract proteins and evaluated for proteasome subunits levels. Western blots of NRK cell extracts indicated decreased levels of 20S proteasome subunits (α3 and β5), after peroxynitrite treatment (Figure 3), suggesting that ROS altered these proteins levels.

Figure 2. *Knockdown of β5 or Rpt6 Subunit of the Proteasome Increases ROS Production in NRK Cells. Normal Rat Kidney Tubular (NRK) Cells were Preloaded with MitoSOX™ Red reagentfollowed by transfection with β5 or Rpt6 sigenome siRNA SMART Pool (100 nM). Equal Concentration of Scrambled siRNA was Used as a Control. The Next Day, Cells were Evaluated for Superoxide Production by Visualizingred Fluorescence Using a Nikon Eclipse E800 Microscope with a Rhodamine Filter Using a Water Immersion Objective (60X). All Images were Captured with Equal Exposure Times and the Fluorescence Intensity was Evaluated Using Image J Software by Calculating Corrected Total Cell Fluorescence (CTCF) (Please Refer to Methods Section). Representative Images of 4 Independent Analyses is Shown and the Values are Expressed as the Mean±SEM (bars) of (n=4). Differences Between Means were Compared with a One-way ANOVA for Multiple Group Comparisons (Scrambled siRNA, β5 siRNA, and Rpt6 siRNA Groups).*

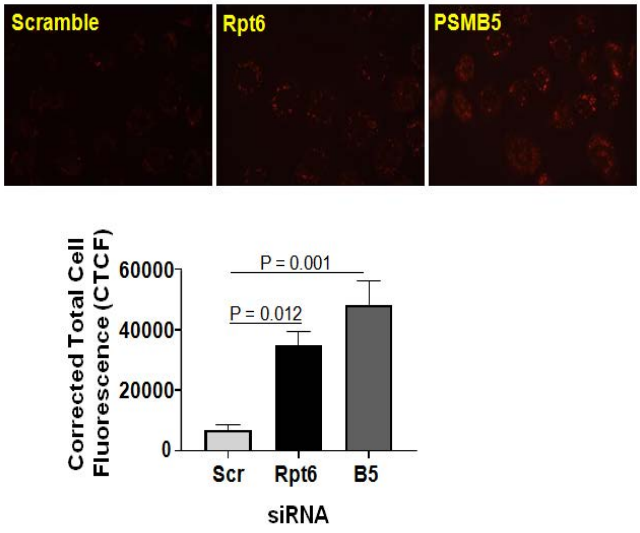
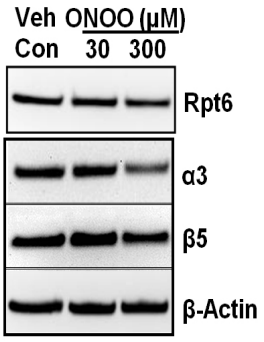


Figure 3. *Peroxynitrite Treatment Impairs Proteasome Subunits in NRK Cells. NRK Cells were Exposed to Peroxynitrite with Dose as Indicated for 18 hrs. Cells were then Harvested and 30 μg of Renal Extracts were Evaluated with Western Blots for Proteasome Subunits (Rpt6, β5 and α3) Levels. β-actin was Used as Loading Control. Representative Western Blots of 3 Independent Analyses is Shown.*



DISCUSSION

Our previous report provided evidence that the functional proteasomes are required to maintain the integrity of mitochondria in kidneys/renal cells, and the proteasome function inhibition by BTZ (in NRK cells) directly impacts the homeostasis of proteins

involved with mitochondrial respiration.[11] Here, we demonstrated that both, pharmacologic (BTZ mediated inhibition) or genetic (siRNA mediated knockdown of β5 subunit) modulation of the proteasome increases mitochondrial ROS in renal proximal tubular cells (Figures 1 and 2). Various doses of BTZ proportionately increased mitochondrial ROS (Figure 1). These studies suggest ROS as a mechanism of disrupted proteasome and mitochondrial function. *In vitro* studies using non-renal cells show induction of antioxidant enzymes following proteasome inhibition .[15-17] In a study by Maharjan S et al, mitochondrial antioxidant (MnSOD) overexpression in Chinese hamster ovary (CHO) cells is shown to be protective against MG132 (a proteasome inhibitor)-mediated oxidative stress and cell death.[18] Collectively, these findings suggest that proteasome appears to be involved in a redox regulation *via* antioxidant mechanisms.

Evidence from *in vitro* models (non-renal) suggests that oxidized proteins are removed by the 20S proteasome.[19-21] ROS are considered critical determinants for proteasome function.[9,10,22] No studies, to our knowledge, have considered the contribution of ROS on 20S proteasome subunits in renal cells. In this report, we provide evidence of the peroxynitrite-mediated decline of α3 and β5 subunits of the proteasome in NRK cells (Figure 3). These results suggest that the declined proteasome function following higher levels of ROS exposure may have resulted from the direct impairment of these subunits (β5 and α3). *In vitro* studies in mammalian cells have shown that dissociation of 20S proteasome from 19S particle occurs following ROS exposure and the levels of 26S proteasome declines with respect to higher doses of ROS.[9 10,22] Future studies are needed to determine the mechanisms of reduction of α3 and β5subunits of the proteasome following ROS exposure.

Emerging evidence suggests that post-translational modifications to proteasome subunits may significantly impact proteasome function.[23-28] Although we did not evaluate any post-translational protein modifications, it is plausible that α3 and β5 subunits could be the targets of oxidative post-translational modifications that alter these subunits and decrease proteasome composition and function in renal cells. It is suggested to evaluate whether post-translational modifications to β5 and α3 subunits of the proteasome reduces levels of these proteins following ROS exposure and that may provide further insight with regard to precise mechanisms of proteasome dysfunction.

CONCLUSION

The maintenance of proteome integrity is essential for renal cell viability during stress. Misfolded or damaged proteins should be monitored by the proteasome, a protein quality-control machinery, which degrades damaged proteins. Here, we demonstrated that the decline of proteasome subunit or inhibition of proteasome subunit results in mitochondrial ROS production. On the other hand, our results show that high levels of ROS decrease the levels of key subunits of the 20S proteasome. Together these data indicate that a cycle of proteasome inhibition and ROS production that could be detrimental to renal cell health during stressful conditions, especially during renal ischemia-reperfusion injury.

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Review

A Novel Hospital-Based Mass Casualty Decontamination Facility for Hazardous Material Disasters

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ABSTRACT

Since the Sarin incident in the subways of Tokyo in 1995, there has been an unprecedented increase in the use of chemical agents on civilian populations internationally. This scourge of chemical terrorism has been relentless worldwide and is likely to continue to be a public health issue that needs to be addressed by the relevant authorities as part of national disaster preparedness and response. One aspect of chemical disasters involves the need for mass decontamination of chemically-contaminated casualties from the scene. The traditional role of hazardous materials civil defence experts in providing such decontamination of victims in the pre-hospital setting is limited by many factors. The presence of congestion in densely populated areas in a highly built up environment of modern-day cities, compounds the timeliness of putting up cordons and crowd control and hence delays the prompt set up of such mobile decontamination facilities close to the incident site. The expected side effect is an almost instantaneous influx of contaminated casualties to the nearest hospital in such situations, which drives the need for public hospitals to be ultimately capable of performing mass casualty decontamination as part of hazardous materials disaster preparedness. This review presents an innovatively designed rapidly deployable hospital-based decontamination facility that has served a tertiary care hospital in Singapore for the last 2 decades in being prepared for managing mass casualties arriving from a chemical disaster in a timely manner.

Keywords
Decontamination; Chemical incident; Industrial disasters; Toxic industrial chemicals; Hazardous materials preparedness; Disaster contingency plans; Emergency preparedness.

INTRODUCTION

Disasters involving chemical release are of concern in our modern industrialized world with highly urbanized cities because of the propensity of such incidents for causing injury and death in large numbers such as the Bhopal disaster[1] in India in 1984. In our present times of prevailing low intensity conflicts and terrorism-linked events, there is an increasing necessity for medical preparedness in dealing with these situations. The Sarin Incident in Tokyo and Matsumoto,[2-4] demonstrated the impact of mass casualty incidents from chemical terrorism using chemical warfare agents released by simple improvised chemical dispersion methods on an unsuspecting civilian population. Most patients from this incident presented promptly to the nearest emergency departments (ED) with no pre-hospital or hospital decontamination resulting in significant secondary contamination of emergency rescue and hospital personnel. Our local experience[5-7] in chemical disasters demonstrated the impact on hospital personnel when over a third of healthcare providers on duty including all responding trauma team members developed secondary symptoms from managing tear gas contaminated casualties with no prior decontamination. Bearing this in mind, all public hospitals in Singapore were tasked by the health authorities to prepare for hazardous materials disasters and had to be equipped to deal with managing contaminated casualties and the decontamination of these victims to reduce toxic exposure to the victims and address the potential spread of contamination to limit damage from secondary exposure of healthcare providers and secondary contamination of healthcare facilities. Most healthcare systems depend on the fire services which predominantly uses the traditional ladder pipe system set up for decontamination of mass casualties.[8] There have been innovative approaches to dealing with this situation including a recent study

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Imamedjian et al[9] trialling the use of public buses and tents as holding areas and decontamination facility. The stark challenges of acute care hospitals in the United Kingdom[10] to be prepared to perform mass decontamination are well-documented from lack of critical infrastructure, logistics, manpower and consistent regular training to maintain competency amongst healthcare providers. In the Singapore context, we have been using our tried and tested rapid deployment hospital decontamination station (HDS) located near to the ED for the last two decades. This article reviews the unique features of this entity.

Unique Features of the Hospital Decontamination Station (HDS)

Singapore General Hospital (SGH), was the first institution in Singapore to take on the challenge to innovate and build the HDS which set the precedent for all other local institutions. The Department of Emergency Medicine (DEM) celebrated its 70th anniversary in 2018. It is an opportune moment to acknowledge one of its pioneer achievement's; the hospital decontamination station (HDS), an important innovative facility the first of its kind in Singapore. The HDS was built in the early part of this millennium and was intended as a medical countermeasure to the rising threat of chemical terrorism and industrial chemical accidents to decontaminate chemically contaminated casualties arriving at the hospital from the disaster scene at short notice. We describe the special considerations that went into constructing the HDS that makes it stand out as a significant countermeasure addressing the unique challenges of dealing with hazardous materials disasters with contaminated casualties by its timely deployment.

Figure 1A. *Ambulance Parking Bay Immediately Outside the Department of Emergency Medicine (DEM) Entrances*

Lessons from the past, both local and overseas, have shown the rapid escalation of chemical disasters with mass casualties. The likelihood of setting up pre-hospital decontamination during disasters by rescue services in most modern-day built up cities which are gridlocked with traffic congestion and high population density is low. It is appreciated that prompt deployment of such decontamination facilities to deal with contaminated casualties who are likely to arrive in large numbers at short notice with no prior decontamination at scene is paramount to successful management of such scenarios. In this regard,the HDS (Figures 1A, 1B and 1C), a semi-automatic decontamination facility located in proximity just outside the entrance of the Emergency Department (ED) that could be operational within 5-minutes of activation by a single staff member is purpose fit. The alternative approach in the past using mobile commercial decontamination systems (Figure 2) took 45-minutes to deploy with specially trained manpower. The space constraints in storing these commercial decontamination sets, retrieving it when required for deployment and short shelf life are additional limitations.

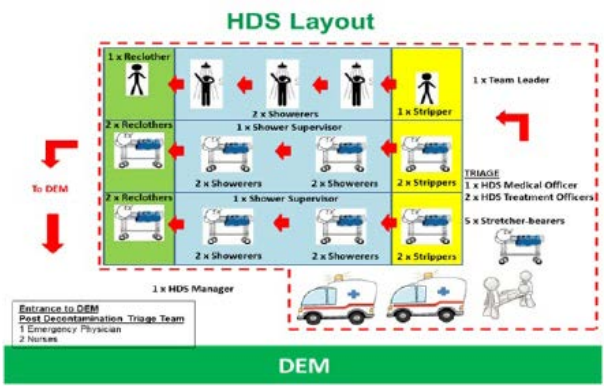
Figure 1B. *Deployment of Hospital Decontamination Station (HDS) Shower Curtains*

Figure 1C. *Hospital Decontamination Station (HDS) in Full Deployment*

Figure 2. *Commercial Mobile Decontamination Shower for Ambulatory Casualties*

The rapidly deployable HDS has capability to decontaminate 42 casualties per hour including 24 non-ambulatory and 18 ambulatory casualties, hence increasing the decontamination capabilities of SGH compared to the commercial decontamination station located off site from the ED which had capability to decontaminate 36 casualties per hour including 6 non-ambulatory and 30 ambulatory casualties. The layout of the HDS is as shown in Figure 3 with 1 ambulatory and 2 trolley shower lanes each divided into 3 enclosed sections beginning with disrobing, showering and re-clothing areas.

Figure 3. *Layout of the Hospital Decontamination Station (HDS) Located just Outside of the Entrance to the Department of Emergency Medicine (DEM)*



There are several unique features of the HDS that are illustrated below:

1. An innovative idea that maximizes use of limited space for dual functions of ambulance parking during daily routine operations and decontamination station during chemical disasters, while preserving aesthetics. There is no need for additional storage space as deployment screens and showers are stowed up on the ceiling with minimum maintenance required (Figures 1A,1B and 1C).

2. Close proximity to ED, which operates round the clock and provides initial manpower to operate the system.

3. Rapid deployment (within 5-minutes of activation) by one staff member operating a control panel with clearly displayed instructions that are easy to follow (Figure 4).

4. Scalable deployment (partial or full) according to caseload depending on small or large scale incident (i.e. the number of lanes deployed can be selected) giving better control over limited space.

5. The enclosed part of the HDS is separated into 3 sections, disrobing area for removal of contaminated clothing, shower section and drying and re-clothing section. This allows for privacy while casualties are undergoing decontamination.

6. Separate sections for ambulatory and non-ambulatory casualties with clear markings on the floor to indicate direction of casualty flow to facilitate work processes.

7. Self-contained disrobing, shower and re-clothing compartment formed by flexible longitudinal screens (premature ventricular contractions (PVC) coated polyester yarn with counterweights and anchors at base to prevent movement) and specially designed lateral cut up staggered screens ensuring ease of movement of casualties and staff working in the HDS.

8. Dedicated, self-contained ventilation system (exhaust fans) which suctions air from within the decontamination station, passing through filters before blowing out to the atmosphere facilitating cooling for staff working with protective suits and reducing contamination within the confined space of the HDS in an environmentally friendly manner.

9. In-built, showers spray heads and soap dispensers deployed from the ceiling to facilitate decontamination, maximize space utilization and ensure decontamination crew safety (Figure 5) by reducing clutter and obstacles which predisposes to fall hazards.

Figure 4. *Control panel for activation of Hospital Decontamination Station with Options for Partial or Full Deployment*

Figure 5. *View from the Trolley Shower Lane within the Hospital Decontamination Station (HDS) Showing Water Hose Deployed from the Ceiling upon Activation*

10. A large holding tank located beneath the HDS to contain the decontamination effluents for subsequent analysis and testing to determine safety for release into the sewerage system ensures water pollution control. This serves to conserve the environment.

Utility of the Hospital Decontamination Station (HDS)

Since its construction, the HDS has been used for several chemical incidents involving industrial chemical contaminated casualties and accidental toxic release situations.[5-7] The rapid deployment capability allowed the incidents to be promptly managed preventing escalation of the incident with good outcomes.

The HDS has also been deployed successfully many times a year for both routine training as well as hospital and national disaster preparedness exercises (Table 1).

Table 1. *Hospital Decontamination Station (HDS) Utilization Over the Years*

Year	HDS Training Courses	HDS Chemical Disaster Exercises
2012	8	1
2013	6	1
2014	10	1
2015	11	-
2016	13	2
2017	18	2
2018	23	2

DISCUSSION

A significant difference between hazardous materials incidents (chemical disasters) and conventional disasters involve the presence of chemical contamination in the environment as well as on casualties arriving from the incident site. This predisposes to ongoing chemical toxicity to the casualty as well as poses added risk to the rescuers and healthcare personnel dealing with such contaminated casualties.

In the Sarin incident in Tokyo in 1995, it was noted that approximately 9.9% of rescue workers[2] (emergency medical technicians (EMT')) and 23% of hospital staff[3] dealing with these chemical contaminated casualties suffered from symptoms due to secondary exposure from chemicals off gassing from clothing's of the casualties. Similar problems with secondary exposures amongst rescue personnel and hospital staff was noted in a similar attack[4] involving Sarin in Matsumoto, Japan in 1994. It is noteworthy that no field or hospital decontamination of casualties was done in both these incidents.

Our own local experience,[5-7] from treating casualties arriving from accidental toxic exposure has reinforced the potential risk of secondary exposure of healthcare workers from hazardous materials and has demonstrated the benefits of timely decontamination.

Hence, in chemical disasters it is of paramount importance to decontaminate all casualties so as to reduce the toxic effects on the casualty as well as to decrease the risk of secondary transmission. The latter is important to reduce the escalation of the event by spread of contamination and to reduce the loss of limited rescue and healthcare resources at a time of increased demand in a disaster situations.[11] The basis for this has been recognized and there is guidance from occupational safety and health administration (OSHA)[12] on best practices to follow in such situations. SGH has adapted and evolved its hazardous materials response contingency plans over time incorporating specific decontamination protocols into their disaster response plans[13] (Figure 6).

Figure 6. *Decontamination Crew Showering Casualty in Shower Section*

There are many recommendations for hospital-based decontamination.[14-20] However, there is limited information on the specific infrastructure required and the necessary manpower and logistical support to execute a successful hospital-based decontamination system. There are many areas that need to be addressed for hospitals to be prepared to deal with hazardous materials mass casualty incidents[21-24] and it is hoped that by sharing our experience on the specifics of one of our crucial assets, others would be able to gain some insights into what it entails and what needs to be done to be ready when the need arises.

CONCLUSION

The HDS marks an important milestone in the hospital's preparedness in dealing with hazardous materials disaster management. It is expected to be the primary decontamination facility for contaminated casualties from the incident site who make their way promptly to the hospitals ED bypassing onsite decontamination. With ED and hospital staff trained in deployment and operations of the HDS it will no doubt be an important countermeasure in the armamentarium for managing contaminated casualties and in the hospital's hazardous materials incident contingency response plans for many more years to come.

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