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Original Research

Immunotoxic Effects of Cypermethrin in Male Wistar Rats: Attenuation by Co-Administration of Zinc and Alpha-Lipoic Acid

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ABSTRACT

Aim
The present study investigated the effects of cypermethrin exposure on humoral and cellular immune response in rat and its attenuation by zinc and alpha-lipoic acid.

Methods
Cypermethrin at the dose levels of 40 mg and 80 mg/kg body weight were orally administered and pre-treatment of zinc (227 mg/L in drinking water) and alpha-lipoic acid (35 mg/kg body wt.) were done. Total leukocyte and differential leukocyte counts (DLC), phagocytic index, serum nitric oxide (NO) activity, total immunoglobulin concentration, quantitative hemolysis, proliferation assay of blood mononuclear cells were estimated and histological examination of spleen was accomplished.

Results
Total white blood cell (WBC) count and percentage of lymphocyte, serum nitric oxide activity ($p<0.001$) and quantitative hemolysis were increased significantly increased whereas neutrophil %, total serum immunoglobulin, and blood mononuclear cell proliferation ($p<0.001$) and the phagocytic function of peritoneal macrophages were significantly reduced in cypermethrin treated rats compared to control group rats at a dose-dependent manner. Zinc and alpha-lipoic acid pre-treatment reversed the results.

Conclusion
From the findings it can be concluded that the co-administration of zinc and alpha-lipoic acid significantly attenuated the immunotoxic effects in cypermethrin exposed rat.

Keywords
Cypermethrin; Zinc and alpha-lipoic acid; Total serum immunoglobulin; Blood mononuclear cell proliferation; Phagocytic index.

INTRODUCTION

Pyrethroids are the second widely used insecticides to control agricultural and indoor pests.[1] Due to wide usage, pyrethroids have been detected in non-target organisms, including fish and human.[2] Cypermethrin, a member of the family of synthetic pyrethroids of type II class, is extensively used in agricultural and other domestic applications. It is well-established that cypermethrin, both cis- and trans-isomers are metabolized to phenoxybenzoic acid and cyclopropane carboxylic acid.[3] Populations at the highest risk of high-dose exposure are producers, hygienic, and pesticide workers, and small farm owners applying cypermethrin for plant

protection. Low-dose exposure originates mainly from the household application of insecticides, contaminated food, and water.[4]

There are substantial evidences that pyrethroids create toxicities apart from their actions in the nervous system.[5,6] Immune-insufficiency of allethrin, cypermethrin, fenpropathrin, permethrin was studied with regard to pyrethroid insecticides.[7] The immune system comprising specialized, memorized complex cells, tissues and organs and they exhibit innate and adaptive responses to protect organisms from different pathogens as well as to maintaining life processes. The relevant interaction between immune response alteration and stress are shown by various epidemiological and experimental studies.[8] Synthetic pyrethroid might induce stress-like symptoms in experimental animals.[9] In immunotoxicological studies with synthetic pyrethroid insecticide cypermethrin, dose dependent suppression of humoral and cell-mediated immune response was induced.[10] It has been reported that pyrethroid insecticides are genotoxic in mouse spleen and bone marrow as well as in cultured mouse spleen cells.[11]

As important trace element zinc controls immune function and cell proliferation.[12,13] Through metallothionein zinc potentiates antioxidant system that impedes oxidative stress facilitated cell injury.[14,15]

By assisting in acyl-group transfer alpha (α)-lipoic acid play role as a coenzyme in the TCA cycle and is considered as a food supplement exhibiting its antioxidant properties. Through the reduction of free radicals it safeguards diabetes mellitus, aging, neurodegenerative and vascular diseases.[16-18]

As the immunotoxicity of synthetic pyrethroid cypermethrin is not well-explored, we have focused our present study on the humoral and cellular immune responses of cypermethrin in a rat model.

According to Goel et al,[19] pretreatment of zinc to chlorpyrifos intoxicated animals significantly improved the blood toxicity (at the dose level of 227 mg/L in drinking water). Andreeva-Gateva et al,[20] evaluated the effect of alpha-lipoic acid (35 mg/kg i.p.) on brain oxidative stress (OS) in unilateral intrastriatal (6-OHDA) injected rats.

The prophylactic effect co-administration of coenzyme Q10 and alpha-lipoic acid was reported in experimentally cisplatin-induced nephrotoxicity of male albino rats.[21] Co-administration of alpha-lipoic acid and vitamin E protect renal cells from injury caused by ROS mediated oxidative stress and related vascular complications induced by nano zinc oxide in rats.[22]

This study also aimed to explore the possible protective role of co-administered zinc and alpha-lipoic acid on any attenuation in immunotoxicity, if any, after oral exposure to cypermethrin in male albino rat.

MATERIALS AND METHODS

Chemicals

Cypermethrin 10% emulsifiable concentrate (EC) named "Ustad" (United Phosphorus Limited, Mumbai, India), Zinc sulphate ($ZnSO_4$), white blood cell (WBC) dilution fluid, chloroform, ethylene-diamine-tetra-acetic acid, phosphate buffer, histopacque-1077, RPMI-1640 and other chemicals were procured from Merck Ltd., Himedia, Mumbai, India.

Animal Maintenance

Male Wistar albino rats weighing 130-150 g were selected for the study. Animals were acclimatized for 10-days before the experiment schedule. Rats were provided standard diet and water sufficiently. They were maintained under 25±2 °C (approximate) temperature and 12 h light-dark cycles throughout the period of experiment. Experimental protocol and surgical methods were reviewed and approved by the Institutional Animal Ethical Committee (IAEC), registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, New Delhi, India).

Treatment Protocol

Thirty-six male Wistar albino rats weighing 130-150 g were randomly assigned to the five experimental groups and one control group, each containing six rats. Groups were designed as: Group I: Control (5 mL/kg body weight), Group II: Zinc (227 mg/L in drinking water) and lipoic acid (35 mg/kg body weight) control, Group III: Cypermethrin-treated (Low dose, 40 mg/kg body weight), Group IV: Zinc and lipoic acid+Cypermethrin-treated (Low dose, 40 mg/kg body weight), Group V: Cypermethrin-treated (High dose, 80 mg/kg body weight), Group VI: Zinc and lipoic acid+Cypermethrin-treated (High dose, 80 mg/kg body weight) group.

Rats were treated orally following the outlined schedule each day at the same time for 14 consecutive days as described in OECD guideline.

On the 15th day, rats were sacrificed, blood samples and the specified internal organ (spleen) from control and treated rats were immediately collected for biochemical, hormonal and histological analysis.

Blood Collection

Blood samples were collected from the treated groups by cardiac puncture. Collected blood was allowed to pour take drop-by-drop into a graduated centrifuge tubes containing ethylene-diamine-tetra-acetic acid (EDTA) for the determination of total leukocyte count (TLC) and differential leukocyte count (DLC).

Total Leukocyte Count

Total leukocyte count[23] was estimated by diluting blood in 1:20 dilution with WBC dilution fluid and total leukocytes were counted in a Neubaur haemocytometer chamber.

Differential Leukocyte Counts

Thin blood smear in a clean glass slide was stained with Leishman's stain and it was observed under the microscope. The percentage of granulocytes and agranulocytes was calculated.[23]

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Collection of Peritoneal Macrophages

The peritoneal macrophages were collected from the animals using i.p. injection of 5 ml of BSA with 5 cold PBS. After 24 h, cells were collected from peritoneal cavity and centrifuged at 1000 rpm for 10 min and it was suspended in RPMI 1640 medium containing 10% fetal bovine serum. Then the macrophage suspension was added to 96-well tissue culture micro-plates microplates at density 1×10^6 cells/well.

Study of Phagocytic Activity

Five hundred (500) µl of the aliquot of cells containing peritoneal macrophages (density of 2×10^6 cells/ml) was mixed with 500 µl of Roswell Park Memorial Institute (RPMI)-1640 containing 10% fetal bovine serum (FBS) and prepared charcoal solution. The mixture was applied to a glass slide and incubated for 2 h at 37 °C in a humid chamber. The phagocytic index was determined by checking the Giemsa-stained phagocytic cells under the light microscope.[24]

Serum Nitric Oxide (NO) Activity

The serum was suspended in phosphate buffered saline (PBS) and it was centrifuged at 10,000 rpm for 15 min. Then the cell-free supernatant was collected and nitric oxide released was measured using the Griess reaction.[25]

Determination of Total Immunoglobulin Concentration

Total serum immunoglobulin was determined by zinc sulfate turbidity test.[26] Briefly, 25 µl of the collected serum were mixed with 1700 µl of 0.7 mM zinc sulfate at pH 5.8. The mixture was shaken and left for 1-hour at room temperature. Serum mixed with PBS at the same ratio was utilized as the blank or control. Optical density was measured spectrophotometrically at 545 nm wavelength.

Quantitative Hemolysis Assay

Quantitative hemolysis assay was done using the method of Simpson and Gozzo with some modifications.[27] One (1) ml of serum was collected and incubated for 3 h at 37 °C. After centrifugation at 3000 rpm for 3 min, the optical density of the supernatants was determined at 560 nm using a spectrophotometer.

Proliferation Assay of Blood Mononuclear Cells

Blood mononuclear cells (BMCs) were suspended in RPMI 1640 medium supplemented with 10% FBS, 100 IU/mL penicillin and 100 µg/mL streptomycin. BMCs (5×10^5) from treated and control animals were cultured for 24 h. After 3 h of incubation at 37 °C in 5% CO_2, the optical density was measured at 450 nm. The proliferation percentage was calculated by dividing each value (tested) by the average mean of the control samples multiplied by 100.[26]

Histological Examination of Spleen

The collected tissues from sacrificed animals were dehydrated in increasing ethanol concentrations, cleared with xylene and embedded in paraffin. Then 5 µ thick tissue sections were cut using microtome and stained with hematoxylin and eosin stain (H&E).

Table 1. *Effect of Zinc and α-lipoic Acid on Total and Differential Leukocyte Count of Cypermethrin-Treated Rat*

	WBC Count /µL	Lymphocyte Count (%)	Neutrophil Count (%)
Control	5591±58	54.33±0.57	35.5±0.428
Zinc and lipoic acid control	5600±64	54 ±0.577	35.5±0.428
Cypermethrin low dose	7275±83a***	63.66±0.666a***	25.5±0.428a***
Cypermethrin low dose+zinc and lipoic acid	5716±83b***	53.66±0.666b***	35.5±0.428b***
Cypermethrin high dose	6866±102a***	64.833±0.477a***	23.6±0.494a***
Cypermethrin high dose+zinc and lipoic acid	5508±58c***	56.5±0.428a*c***	29.5±0.428a*c***

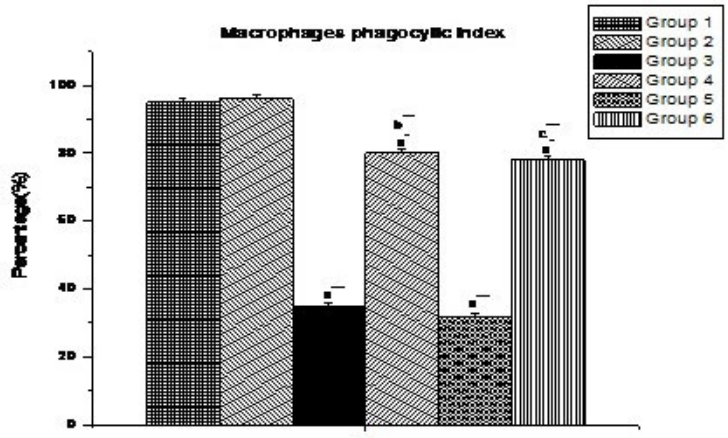
Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; (indicates p<0.01; *** indicates p<0.001)*

Table 2. *Effect of Zinc and α-lipoic Acid on Total Immunoglobulin conc. (g/L), Quantitative Hemolysis (%) and BMC Proliferation (%) of Cypermethrin-Treated Male Albino Rat*

	Total Immunoglobulin con (g/L)	Quantitative Hemolysis (%)	BMC Proliferation (%)
Control	5.12±0.21	0.134±0.001	85±1.5
Zinc and lipoic acid control	5.02±0.25	0.136±0.002	82±2
Cypermethrin low dose	3.02±0.22 a***	0.189±0.002a **	50±1.5a**
Cypermethrin low dose+zinc and lipoic acid	4.48±0.12 a*b***	0.131±0.002b**	72±1.2a*b**
Cypermethrin high dose	3±0.23a***	0.198±0.003a**	38±1.2a***
Cypermethrin high dose+zinc and lipoic acid	4.52±0.24 a*c***	0.135±0.001c**	65±1.5a*c**

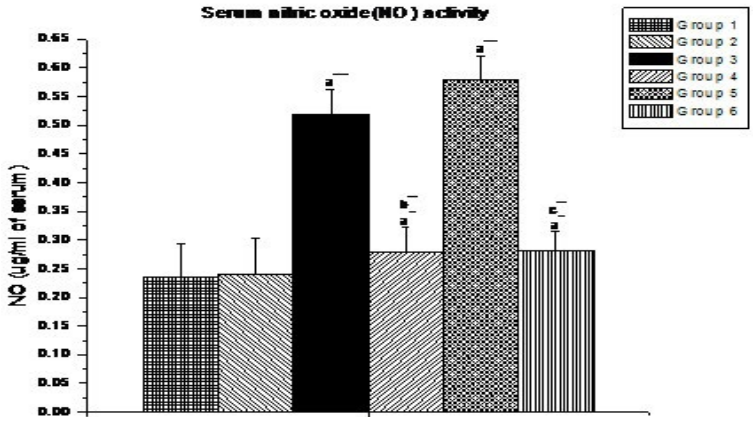
Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; (indicates p<0.01; *** indicates p<0.001*

Figure 1. *Illustrates the Effect of Zinc and α-lipoic Acid on Macrophage Phagocytic Index in Cypermethrin Induced Male Albino Rat*



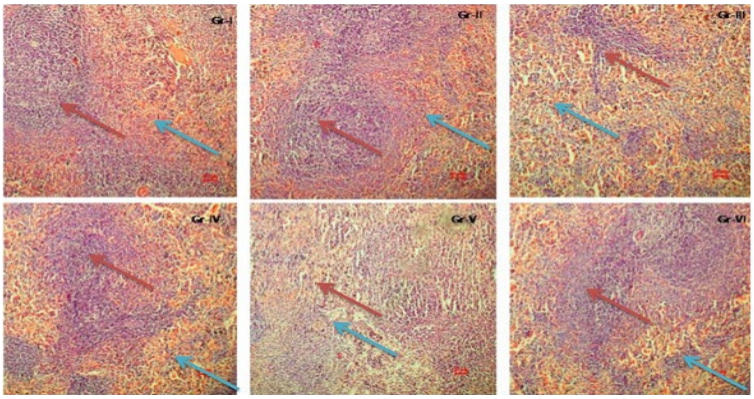
*Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript b Group-III vs Group-IV; Superscript c Group-V vs Group-VI. Asterisks represents the different level of significance (** indicates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

Figure 2. *The Effect of Zinc and α-lipoic Acid on Serum Nitric Oxide Activity in Cypermethrin Induced Male Albino Rat*



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

Figure 3. *The Effect of Zinc And α- Lipoic Acid on Splenic Histology of Cypermethrin Induced Male Albino Rat*



Red arrow indicates red pulp; blue arrow indicates white pulp. Group I shows normal red pulp and white pulp also group II shows normal red pulp and white pulp. Group III shows disintegration of red pulp and white pulp. In Group IV we find out the normalization of white pulp and red pulp. But in Group V red pulp and white pulp are not clear. But Zinc and alpha lipoic acid has tried to normalize the histology. Bar scale 10 µm. Magnification 200X.

Images of the histological sections were analyzed using light microscopy.

Statistical Analysis

The results were expressed as the Mean±Standard error of mean (SEM). Statistical analysis of the collected data was performed by analysis of variance (ANOVA) followed by two-tail *t*-test. The difference was considered significant when $p<0.05$.

RESULTS

Effect of Cypermethrin on Rat Leukocytes

Table 1 presented that the total WBC count and percentage of lymphocyte were significantly increased ($p<0.001$) as well as neutrophil % ($p<0.001$) were significantly reduced in low- and high-dose of cypermethrin treated rats compared to control group rats. zinc and lipoic acid co-administration ameliorate the total WBC count and neutrophil percentage ($p<0.001$).

Effect of Cypermethrin on Phagocytic Index

Figure 1 showed that phagocytic index (macrophage) in both low- and high- dose cypermethrin treated rats were significantly diminished ($p<0.001$) in comparison to the control group animals and pre-treatment with Zinc and lipoic acid significantly ($p<0.001$) increased the phagocytic index of macrophages.

Impact of Cypermethrin on Nitric Oxide Activity

Pre-treatment of zinc and lipoic acid resulted in a significant decrease ($p<0.001$) in serum nitric oxide activity which were significantly increased ($p<0.001$) in the low- and high- dose of cypermethrin treated animals compared to control groups rat (Figure 2).

Effect of Cypermethrin on Total Immunoglobulin, Quantitative Hemolysis, Blood Mononuclear Cell Proliferation

As shown in Table 2, cypermethrin reduced total immunoglobulin concentration and blood mononuclear cell proliferation ($p<0.001$) whereas quantitative hemolysis was significantly increased ($p<0.001$) in cypermethrin treated rats and co-administration of zinc and lipoic acid returned it towards normal levels.

Effect of Cypermethrin on Splenic Histology

Figure 3 showed marked alteration in splenic red and white pulp area in low and high dose cypermethrin treated animals. In zinc and lipoic acid pretreated rats, the architecture of the spleen was altered towards normal.

DISCUSSION

The acute toxicity of many pesticides is well-known and poisoning cases are often reported. In contrast, much less is known about long-term impacts on human or different animal systems including the nervous, hormone, reproductive and immune systems. Immunotoxicity of pyrethroids have been reported earlier by several researchers.[28,29] Immunosuppressive effects associated with high doses of deltamethr in[28] and fenvalerate[30] on humoral and cell-mediated immune responses in different species like adult mice, rats, and goats have been reported.

The present study may be considered as a part of subacute toxicity study and reports the alterations of immunological parameters and the beneficial effects of zinc and lipoic acid treatment in cypermethrin intoxicated male rats.

The increased leukocyte (WBC) counts were noted in cypermethrin treated groups and it may be due to the activation of the defense and immune systems of the body.[31] This may results in an increased release of WBC from the bone marrow storage pool into the blood. The primary function of white blood cells is to defend against foreign bodies, which is attained by leucocytosis and antibody production. Pathological leucocytosis may occur due to exposure of chemicals or acute haemorrhages and haemolysis. Leucocytosis may be raised due to resistance of the animal for localization of the inflammatory response. Another possible cause of leucocytosis may be the severe haemorrhages in liver and lungs.[32] This increase may be related to an increase in lymphocyte percentage. These results indicated that zinc and lipoic acid might have a beneficial role in lowering pyrethroidstoxicity probably due to its radical scavenging property.[33]

On the contrary, neutrophil counts were found to be decreased after 14-days of cypermethrin treatment. Neutrophils act as defence cells against foreign materials[34] It is usually recognized that the innate immunity actions of neutrophils are generally facilitated by phagocytosis, discharge ofgranules, and development of neutrophil extracellular traps (NETs).[34] It may be due to decreased immunity for the increased cypermethrin intoxication. Simultaneous co-administration of zinc and lipoic acid to cypermethrin treated animals improved the altered levels of haematological parameters. Interestingly, zinc and lipoic acid pre-treatment to cypermethrin intoxicated rats restored the levels of total as well as differential WBC count to the normal levels. These observations might also indicate that zinc and lipoic acid have therapeutic and beneficial effects on cypermethrin-mediated toxicity.

Phagocytes detect infected microbial pathogens and activate innate immune responses and it is crucial for maintaining or restoring host homeostasis.[35] Macrophages as a phagocyte have key roles in host defense against microbes mediated infections by eliminating the pathogens producing modulation of immune responses. Both macrophages and neutrophils are conscripted to the inflamed site from circulating blood during microbial infection to notice, exterminate, and engulf the invading microorganisms. Significant inhibition in humoral immune response was detected in animals treated with high- and low- dose of cypermethrin. The detected reduction of humoral immune response assured the immunosuppression demonstrated after exposure to type II pyrethroids in the murine model. Nonspecific cellular immune response that examined in the current study showed significant inhibition in the phagocytic activity of peritoneal macrophages in cypermethrin-treated rats as compared to the control animals. Zinc and lipoic acid pretreatment ameliorate the toxicity.

Increased NO level designates adiminished antioxidative defence mechanism in arsenic-$NaAsO_2$-induced immunotoxicity *in vivo*.[36] To investigate the changes of immune status, we measured the levels of nitric oxide in the serum. As shown in Figure 2, exposure to cypermethrin was related to an increase in the level of nitric oxide level compared to the control group. Co-administration of zinc and alpha lipoic acid significantly decreased the level of nitric oxide in the serum compared with animals treated with cypermethrin alone.

In the present study a decrease in total immunoglobulin concentration in cypermethrin treated rat was detected. Previous findings indicated that most of type II pyrethroids (cypermethrin, super-cypermethrin forte, fenvalerate, deltamethrin and lambda-cyhalothrin) are known to cause impairment and suppression of immune system in adult rats and rabbits.[10,37] In the cypermethrin treated groups, a significant reduction in total immunoglobulins concentration was detected, which may indicate diminished B-lymphocyte function with the resultant decreased antibody production.[38]

The histopathological findings of the present study revealed that the presence of immunotoxic effects in cypermethrin treated animals. The noticeable alteration in red and white pulp area was observed in the cypermethrin treated rat spleen. The congestion witnessed in the spleen may be as a result of intrasplenic damage of erythrocytes.[39] These findings are in agreement with previous studies[39] where exposure of permethrin adversely affected the function of spleen and thymus, important immune organs.

Zinc influences multiple facets of the immune system.[40] Zinc is essential for maintenance of macrophages, neutrophils, and NK cells and development of cells mediated innate immunity. Zinc deficiency severely affects phagocytosis, intracellular killing, and cytokine production as well as the growth and function of T- and B-cells. Zinc as an antioxidant stabilizes membranes possibly by preventing free radical-mediated inflammatory processes.

Alpha lipoic acid, a natural ingredient of human body, not only acts as a powerful antioxidant but also is able to regulate the immune system in either direct or indirect ways.[41] ALA is used to treat autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, and primary vasculitis.

Co-administration of zinc and alpha lipoic acid significantly attenuated cypermethrin induced immunotoxicity in male Wistar rat probably for their above said properties.

CONCLUSION

Thus, from the findings we may conclude that cypermethrin caused prominent alterations in haematological parameters as well as immunotoxicity in male Wistar rat by impairing the immune status of the body. From the above findings, it is evident that zinc and alpha-lipoic acid have potent ameliorative role on cypermethrin induced immunotoxicity due to its antioxidant and immune status protecting properties.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Case Report

Autonomic Dysreflexia: Atypical Complication from Immediate Release Tapentadol

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ABSTRACT

Neurological disorders are a ubiquitous part of our lives, and with innovative technological advancements there are increasing numbers of people being diagnosed with a variety of conditions. While these advances uncover the underlying pathological process, the requisite need to manage a patient's condition necessitates renewed vigour in the realm of key therapeutics. This case study looks at a patient with a rare neurological condition, transverse myelitis (TM), and a complication that many spinal cord injury patients suffer, autonomic dysreflexia (AD). However, what makes this case unique is when the patient was administered with immediate-release Tapentadol, a synthetic opioid, the patient suffered more frequent and prolonged attacks of AD. The exploration of the functional anatomy of TM as it applies to this case is highlighted, and how the role of Tapentadol was a causative agent in increasing the patient's AD.

Keywords
Spinal cord injury; Transverse myelitis; Pharmacokinetics; Opioids.

Abbreviations
TM: Transverse myelitis; CNS: Central nervous system; AD: Autonomic dysreflexia; VAS: Visual analogue scale; MET: Medical emergency team.

OVERVIEW

Transverse myelitis (TM) is an uncommon acquired neuro-immune spinal cord condition, characterised by inflammatory responses that can manifest with an acute or subacute progression of weakness, sensory deficiency and autonomic dysfunction.[1-3] TM has many and often varying aetiologies, chief among them a post-infectious complication, all the way to idiopathic. Nevertheless, irrespective of the underlying cause, the result for the patient is that the once normal flow of signals in the central nervous system (CNS) undergo a demyelination process, because of the inflammatory and autoimmune response.[2] As each case of TM will differ from case to case, each TM patient is unique, and a reminder of the basic principle that the anatomical structure governs physiological function.

While there is a wealth of literature available on the more common neurological disorders, TM is a rare neuro-immune condition, and it is beyond the scope of this case report to delve into all facets of the condition. However, the exploration of the complication of autonomic dysreflexia (AD) will be examined. First, through a clinical anatomy lens, we survey the underlying aetiology and pathophysiology borne by normal *versus* abnormal anatomy. Second, we explored the effects of analgesic pharmacological agents on the anatomical areas we examined earlier. Ultimately, applying functional anatomy and pharmacokinetics through our case study, we intend to better understand how TM and adverse reactions, such as AD, can impact treatment protocols, through documentation of this research.

CASE REPORT

In this case study, we discuss a 38-year-old male with TM presenting to the hospital emergency department, *via* paramedic assistance due to a sudden and insidious onset of AD. The patient

had undergone recent laparoscopic surgery to remove a necrotic piece of bowel tissue, on the external aspect of the junction of the descending and sigmoid colon, 48-hours prior. The working diagnosis prior to imaging was diverticulitis, as the patient presented with a fever of 40 °C, abdominal tenderness, nausea and a visual analogue scale (VAS) score of 9 out of 10 for pain. Diagnosis, following imaging and at the time of surgical excision was epiploic appendagitis. The patient was discharged a few hours following the procedure, with prescribed rest and oxycodone for breakthrough pain relief. Upon returning home, the patient's demand for pain relief developed into pre-operative pain, and 10 mg of oxycodone was administered orally every six-hours. This pattern continued until the onset of the AD, and the call to the paramedics.

Autonomic dysreflexia is a loss of harmonised autonomic responses, which result in amplified sympathetic responses to stimuli below the level of spinal cord damage, as is the case with our TM patient, leading to turgid vasoconstriction and hypertension.[4] The patient's lesion due to the TM was located at the spinal cord level of T6, and due to the surgical intervention and ongoing irritation in his left iliac region, a region below T6, a cascade of neural signals was sent up the spinal cord and set the AD in motion. This is due to the lesions at T6 not allowing for the signals of the parasympathetic nervous system to counterbalance the influx of sympathetic overflow,[5] causing hypertension, flushing of the face, nasal congestion, thumping headache, piloerection, sweating above T6, cool and damp skin below T6. By the time the paramedics had arrived on the scene, the patient had started to convulse and had a seizure. He was treated *en route* to the emergency department with glyceryl trinitrate sublingual spray (0.4 mg/spray) and given a nasal dose of 0.1 mg of fentanyl, twice. The patient was stabilised in the emergency department and transferred into the hospital wards for observation, and to monitor his pain levels. Under the guidance of a consultant anaesthetist and pain specialist, the patient's medications were carefully monitored and oxycodone was ceased and replaced with Tapentadol: 50 mg sustained-release bd (twice a day), and 50 mg immediate-release qid (four times a day). While Clonidine was titrated up to 100 mg tid (three times a day), from bd and 180 mg of Diltiazem was prescribed by a consultant cardiologist as a prophylactic measure for the patient's hypertensive episodes, following a coronary angiogram to rule-out serious cardiac issues.

Within a day of commencing the Tapentadol course, the patient's episodes of AD began to increase in number and each progressive episode lasted longer. On one of the episodes the patient lost consciousness and a medical emergency team (MET) was called over the hospital emergency system. Upon regaining consciousness, the patient did not recall the episode or the events leading up to the MET call. Cardiac monitoring was subsequently used for a period of 96-hours. When a dose of the Tapentadol 50 mg immediate release was administered orally to the patient, within a 30 to 60-minute period, he would become hypertensive and start having an attack of AD. Oral glyceryl trinitrate (0.4 mg/spray) was administered when the monitors picked-up a spike in blood-pressure raising anything beyond a systolic level of 150 mmHg. During the peak of his AD episodes, monitors recorded hypertension at 240/130 mmHg and a cardiac beat per minute at 148. When the cardiologist and pain specialist reviewed the output from the monitor, they removed the Tapentadol immediate release from the treatment schedule and replaced it with Buprenorphine 0.4 mg of sublingual tablets tid. The patient's episodes of AD subsided over the following days, and he was discharged home, with a follow-up consultation with both specialists within the month.

DISCUSSION

Due to the pervasive nature of laparoscopic surgery to correct a piece of necrotic tissue in the left lower bowel region of the TM patient, while facing the consequence of sepsis, and having pain level increase pre- and post-surgery, the discharge procedure should be questioned in this complex case. Serious abdominal complications, including but not limited to gastrointestinal bleeding, gall stones, or appendicitis have previously raised concerns[4] related to AD and abdominal procedures for patients with spinal cord injuries at or above T6. The pathophysiology for AD to occur is due to the major splanchnic outflow, T6 to L2, which becomes disconnected from supraspinal control. Stimulation in the dorsal and spinothalamic tracts above the lesion level causes the intermediolateral column neurones to become activated by the collateral branches, causing norepinephrine spillover, resulting in hypertension, which can activate baroreceptors that induce vasodilation and bradycardia by the vagus nerve.

The activation of the body's sympathetic nervous system prepares an individual for a "fight-or-flight" response,[6] in preparation for heightened levels of activity to vital organs that require an increased chance of enduring a threat or confrontational situation. Such increases include the dilation of pupils to increase vision, constriction of blood vessels to areas the body deems not under threat, such as the skin or digestive tract, and diversion of more blood to skeletal muscles; dilation of bronchi in the lungs, thus increasing the capacity for oxygenation; increase in cardiac output increases, as the blood vessels around the heart dilate and the heart rate increases; and simultaneously, the release of epinephrine and norepinephrine that is stimulated by the adrenal medulla into the bloodstream. To initiate this physiological cascade of events, the CNS houses, in the spinal cord throughout the thoracic region and the upper two lumbar spinal segments, an arrangement of preganglionic neurones in an area of the cord known as the intermediolateral cell column (or grey matter), within the lateral horn.[7] The axons commencing in the intermediolateral cell column in the thoracic cord, the preganglionic cells, range a short distance to the sympathetic chain ganglia, running parallel and adjacent to the thoracic vertebrae: these are the primary source of sympathetic neurones of the autonomic nervous system which give rise to the fight-or-flight mechanisms to the body: smooth muscle, cardiac muscle and glands.[6] Additionally, there is a subdivision within the thoracic preganglionic fibres in the visceral nerves that course through to the adrenal medulla known as the splanchnic nerves are generally considered for having modified endocrine functioning, namely, the secretion of catecholamines into the bloodstream.

To counteract this overstimulation by the sympathetic nervous system, the parasympathetic nervous control is located predominantly throughout the brainstem region and travels through cranial nerves, primarily with the vagus nerve. As this is

above the level of T6, where the patient's lesion is found on the spinal cord due to the TM, neural signals descending the spinal column to attenuate the activity of the sympathetic overload, and AD, were unsuccessful in reaching their desired location. Therefore, pain signals being directed post-surgically from the patient's bowel triggered the AD by sending repeated pain signals to the spinal cord. This set out a chain reaction by the sympathetic nervous system, compounded by the addition of the opioid pain medication, and the lesion at T6. Thus, the inability for the signals from the brainstem to stabilise this reaction back to a homeostatic state caused the repeated sequence of attacks of AD.

The exploration as to the differences between the Tapentadol immediate release and sustained release will now be explored, as there is scant literature on patients with TM being treated with Tapentadol, and suffering from repeated attacks of AD.

As opioid use is well tolerated and established in an acute pain scenario,[8] semi-synthetic and synthetic opioid pharmacological agents have become established in the past decade, and one such example is Tapentadol. Tapentadol is the most recent of the synthetic opioids to become widely distributed.[9] Typically, opioids are a potent analgesic, as they exert their major pharmacologic effects on the CNS.[9] The effect of not losing consciousness is the underlying clinical benefit to utilising opioids as a therapy, while the analgesia may be accompanied by feelings of exultation, drowsiness or a transient cognitive decline.[8] What makes opioids unique is their ability to bind to specific receptor sites, of which there are three: μ (mu), κ (kappa), and δ (delta). For this case report, we will concentrate on the μ receptor, as Tapentadol's pharmacokinetics relies on this receptor binding site.[10] The pharmacologic profile of the μ receptor is that it generates CNS depression, respiratory depression, miosis, euphoria, a reduction in digestive motility, hypothermia, bradycardia, and physical dependence and tolerance.[9] Tapentadol is a centrally-acting analgesic, which has been synthetically prepared to combine two mechanisms of action: act as a μ-opioid receptor and noradrenaline uptake inhibitor.[10] The drug was approved for use in the United States in 2008,[9] in 2011 for the Australian market,[11] where the current case study is being reported. Tapentadol was available in two forms, a sustained release oral tablet and an immediate release oral tablet in a variety of concentrations.[10]

In a post-marketing study carried out by the drug manufacturer Grünenthal GmbH,[10] the overall safety and adverse drug reactions of Tapentadol were analysed for reported cases, globally. The most prevalent side-effect was nausea in their systematic review for all patients grouped together. Other side-effects included: dizziness, headache, drug ineffectiveness, hallucination, vomiting, somnolence, feeling abnormal, hyperhidrosis, fatigue, confusion, constipation, dyspnoea, and pain.[10] Of note, there were no reports of anything akin to AD, however, the authors did note that these were the reported events and there was the possibility that there may be unreported side-effects.

As this case report centres around the drug Tapentadol in its two forms, immediate-release and sustained-release, it is important to distinguish between them and ascertain why they had such a profoundly divergent outcome on the patient. Both drugs are designed to act by undergoing phase I metabolism by N-demethylation and alkyl hydroxylation,[9] and their agonistic behaviour to bind to a μ-opioid receptor, and inhibit the reuptake of norepinephrine, thus increasing the blood levels of norepinephrine. This is where the distinction occurs, the benefits to extending the release of the active ingredient, Tapentadol into the bloodstream and inhibiting the reuptake of norepinephrine, attenuates adverse effects, while sustaining bloodstream levels for a longer period, rather than in a short burst, which is the case with the immediate-release.[12,13] When a dose of immediate-release is ingested, rates of absorption are sped-up. Thus, the availability of the active ingredients are available within 30-minutes to 1-hour, and its peak levels are at 1.25 hours.[14] In contrast, sustained-release Tapentadol reaches its peak between 3 to 6-hours.[14]

This extreme overload on the patient's body and the ongoing attacks of AD can be attributed to the functions of the hormone norepinephrine, where an increase in heart rate and force of contractility occurred, and the diversion of blood to skeletal muscles, as the vasoconstriction to non-vital visceral organs and skin (in a fight- or-flight situation), and hypertension resulted from the vasoconstriction of systemic blood vessels. This phenomenon was described earlier as the fight-or-flight response of the body, or the sympathetic nervous system getting ready to go into battle or run away from danger. Unfortunately for the patient, his lesion was located at T6 and safeguard mechanisms to shut down or reverse this process were blocked from getting the message through the neural cabling system, the spinal cord.

CONCLUSION

Patients with complex or rare medical conditions provide the medical fraternity with a platform to allow the profession to not only acquire a growing body of knowledge, and establish frameworks for which healthcare providers are equipped with the training, and experience to be able to recognise medical emergencies sooner for the benefit of these patients. The above case study highlights the importance of a working knowledge of applied anatomy as a fundamental and underpinning the very crux of diagnostic rapidity, but also exactitude. TM is a serious neurological disorder, which can have effects on varying functions, dependent on the location of the patient's lesion. For many people with spinal cord injuries above the level of T6, unfortunately the perils of AD are all too familiar; however, if the injury is below this level this neurological complication is not feasible. The addition of pharmacologic agents to disrupt AD has been advantageous in its treatment, yet, it is also these drugs that can intensify the signs and symptoms of AD within moments of ingesting them. With the cessation of immediate-release Tapentadol in this specific case, the patient no longer suffered from attacks of AD.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONSENT

The authors have full consent of the patient to participate in this

case report, and retain their full informed consent form.

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Original Research

Vitamin C, E and Zinc Ameliorates Cadmium-Toxicity Induced Biochemical Changes in Male Albino Rats

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ABSTRACT

Background
Environmental toxicants have become a major source of health hazards to humans, thereby negatively impacting the health and overall well-being of exposed individuals. Among these environmental toxicants, heavy metals stand out as the major cause of tissue pathologies and threaten an individual's health status. One such heavy metal is cadmium (CD) whose exposure has been linked to various tissue toxicities including nervous, respiratory, reproductive, cardiovascular, hepatic and renal tissues. Cadmium is a non-biodegradable heavy metallic which possesses a long half of lifestyles and comfortably accumulates inside the tissues in which it produces tissue toxicities main to tissue disorder. The present study was aimed to determine the amelioration capabilities of Vitamin C, E and Zinc from the harmful effects of CD in Wistar rats.

Methods
The Wistar strain male albino rats weighing 225±10 g were administered with CD along co-administered with Vitamin C, E and Zinc, individually and also in combinations. After the completion of 45-days of experimentation, certain specific enzymatic parameters were assayed in plasma serum to assess the impact of CD and protective effect of Vitamin C, E and Zinc.

Results
Soon after the co-administration of CD along with Vitamin C, E and Zinc, either individually and in combinations, Body weights, liver weight and histo-somatic index (HSI) of liver and certain specific enzymes of plasma including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatinine, glucose and urea were monitored. All the parameters monitored showed a significant (p<0.05) increase during CD administration except ALP. All the parameters selected in the present study were shown to be significantly (p<0.05) reversed due to co-administration of Vitamin C, E and Zinc either individually or in combination, due to the protective effect from CD toxicity in wistar rats.

Conclusion
Our results demonstrate that co-administration of Vitamin C, E and Zinc ably protects the toxicity of CD in Wistar rats significantly.

Keywords
Cadmium; Wistar rats; Oxidative stress.

INTRODUCTION

Over the years, anthropogenic activity have led to accumulation of several heavy metals in the environment, causing serious health problems in humans. Cadmium (CD) is considered one of the most common and most dangerous environmental pollutants of the natural and occupational environment in industrialized countries all over the world.[1] Continuous CD release from natural sources, i.e., the Earth's crust, and various human activities, like industrial processes, has led to an increase in CD concentrations in the environment. Increased CD exposure has increased throughout the population for the lifetime of the individual. Heavy metals exhibit a density that is at least five times greater than that of wa-

ter and isn't biodegradable. The long biological half-life leads to an accumulation in different organ systems, leading to undesirable side effects and are relatively more toxic even at very low concentrations.[2] CD is a heavy metal widely utilized in industry, affecting human health *via* occupational and environmental publicity.[3] CD may be absorbed and accrued in flora and animals through water, air, and soil and, consequently, within the human body through the food chain and is a major source of human exposure with the liver and kidney affected.[1,3] Studies have shown that exposure to CD causes haematological, hepatotoxicity, neurobehavioral parameters disorders. CD-related disorders are associated with CD toxicity in liver and affect cell proliferation, differentiation, apoptosis, and other cellular activities. Cellular changes include the swelling of hepatocytes, fatty changes, focal necrosis, and hepatocytes degeneration, all considered markers of impaired function.[3] The role of certain metals and vitamins in modulating the effects of different toxicants is an area of recent interest. Vitamins are essential to maintain normal metabolic processes and homeostasis within the body. Vitamin C and Vitamin E are low molecular mass antioxidants that scavenge or quench free radicals.[4]

Natural antioxidants, Vitamins A, C, E and carotenoids, are derived from food and are consumed through diet.[5] Recently, much interest has been given to the role of natural antioxidants as prevention against oxidative damage as a factor in the pathophysiology of various health issues.[6] Among the antioxidants ascorbic acid (Vitamin C) and Tocopherol (Vitamin E) used as a nutritional supplements and are considered essential elements in almost all biological systems. Vitamin C is a water-soluble chain-breaking antioxidant and can scavenge superoxide and hydrogen peroxide.[7,8] Vitamin E (α-Tocopherol) is a lipid soluble vitamin with powerful biological antioxidant.[7,9] Zinc is an essential trace element in men, relatively non-toxic,[10] ubiquitous in subcellular metabolism and an integral component of catalytic sites of enzyme classification.[11] To date, the full impact of the environmental contamination has not been elucidated in the biological, where the effect of heavy metals including CD either individually or in combination with natural antioxidants may require a thorough examination to understand the interaction between CD with antioxidants. The present study was performed to determine the amelioration capabilities of Vitamin C, E and Zinc in wistar rats from CD-induced toxicity.

MATERIALS AND METHODS

Animals

Male rats weighing 225±10 g were selected in the present study and were housed in stainless steel mesh cages, under standard laboratory conditions (Temperature 23±2 °C, 50±10%; Relative humidity, 12:12 Light: Dark cycle). The animals were fed with standard rat chow (obtained from Sai Durga Feeds and Foods, Bangalore, India) and drinking water *ad libitum*. The rats were acclimatized to the laboratory conditions for ten days. The Institutional Animal Ethics Committee has approved the Experimental protocols and animal use (Resol. No. 60b/2012/(i)/a/CPCSEA/IAEC/SVU/MSR–RS dt. 08.07.2012), Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Chemicals

Cadmium as Cadmium chloride ($CdCl_2$), Zinc as Zinc chloride ($ZnCl_2$), Vitamin C (Ascorbic acid), and Vitamin E (α-Tocopherol) was obtained from Sigma Chemical Co, Loba Chemicals and SD Fine-Chemicals, Maharashtra, India. All the chemicals used in this study were of the highest purity.

Experimental Design

Rats were divided into 8 groups; each contained 6 rats and fed one of the following diets.

Group 1: Control group
Group 2: $CdCl_2$ dissolved in drinking water @ 10 mg/L
Group 3: $CdCl_2$ (10 mg/L of drinking water)+Vitamin C (100 mg/kg BW)
Group 4: $CdCl_2$ (10 mg/L of drinking water)+Vitamin E (100 mg/kg orally)
Group 5: $CdCl_2$ (10 mg/L of drinking water)+Zinc (15 mg/kg oral administration in drinking water)
Group 6: $CdCl_2$ (10 mg/L)+Vitamin C (100 mg/kg BW)+Zinc (15 mg/kg)
Group 7: $CdCl_2$ (10 mg/L)+Vitamin E (100 mg/kg)+Zinc (15 mg/kg)
Group 8: $CdCl_2$ (10 mg/L)+Vitamin C (100 mg/kg BW)+Vitamin E (100 mg/kg)+Zinc (15 mg/kg)
Quantity of food consumed by rat; 35-60 g forage/day and of drinking water 25-40 mL/day.

After completing the study, all the animals were anesthetized, all the animals were anesthetized and blood samples were collected through cardiac puncture. Animals were sacrificed by cervical dislocation and the rats' liver was removed. Serum samples were separated by using centrifugation at 2000 rpm for 20 min. Serum samples were used for biochemical analysis. The liver was weighed to their nearest mg using Shimadzu Electronic Balance and was used for experimental purposes.

Biochemical analyses were performed by following methods:

The organ weight was presented as relative organ was calculated as follows:

$$\text{Relative organ weight: } \frac{\text{Absolute organ weight (g)}}{\text{Whole body weight (g)}} \times 100$$

Aspartate aminotransferase (AST) (EC: 2.6.1.1): Reitman et al[12]
Alanine aminotransferase (ALT) (EC: 2.6.1.2): Reitman et al[12]
Alkaline phosphatase (ALP) (EC: 2.3.1.1): Rosalki et al[13]
γ-Glutamyl transpeptidase (GGT) (EC: 2.3.2.2): Novogrodsky et al[14]
Lactate dehydrogenase (LDH) (EC: 1.1.1.27): Kornberg[15]
Urea: Patten et al[16]
Creatine: Faulkner et al[17]
Protein: Lowry et al[18]
Glucose: Roe[19]

Statistical Analysis and Data Presentation

All the obtained data were statistically analyzed using SPSS package. Results obtained were presented as Mean±SD for comparison of different experimental animal groups with control ones. The results were statistically analyzed by a one way ANOVA. *p* value<0.05 was considered significant. Data of biochemical measurements were further subjected to estimation of percent of changes caused by exposure to the heavy metal CD and the improvement achieved by co-administration of Vitamin C, E and Zinc amelioration index (AI).

RESULTS AND DISCUSSION

In the present study eight groups of rats were maintained for a period of 45-days. No mortality occurred during the experimental period.

Body Weights and Relative Liver Weights

Final body weights and relative liver weights of male rats subjected to different experimental treatments were obtained and presented in Table 1. The Final body weights were significantly decreased -36.68% (p<0.05) with CD-treated rats compared to control and other co-treatment groups Vitamin C, E and Zinc with CD individually combinations with CD groups. The final body weights were 256.56 g in the control group, and 143.18 g with CD treated group. In contrast, co-treatments with Vitamin C, E and Zinc recorded the body weights 233-238 g range, but the co-treatments with combinations yielded 242-246 g. Among the treatments, all the groups recorded a weight gain (NS) except in CD treated group (p<0.05) which is significant. Ameliorative index calculated recorded to be 62.82, 66.47 and 65.64% with groups pretreated individually with Vitamin C, E and Zinc with CD, respectively compared to only CD treated group which is highly significant (p<0.05). But the combination of Vitamin C, E and Zinc with CD, the Ameliorative index was 69.09%, 70.03% and 72.35%, respectively and are significant (p<0.05) when compared to CD treated group of rats. The body weight gain was 31.28, 8.02, 14.16 and 11.84 g compared to the control group (p<0.05), and with pretreated Vitamin C, E and Zinc with CD groups individually, respectively which are statistically not significant (NS). In contrast, the body weight gain was 16.79, 17.27 and 20.98 g compared to the pretreatment groups of Vitamin C, E and Zinc, combined with CD treatment (p<0.05). But the CD treated group recorded a loss of 32.95 g during experimentation (p<0.05). Liver weights decreased in all experimental groups (p<0.05) with the CD treated group and pretreated Vitamin C, E and Zinc. The rate of change for the reported reductions in weight was not different from the pretreatment groups (Vitamin C, E and Zinc) combined with CD. The histo-somatic index (HSI) of the liver was approximately 3.0 for all groups.

Liver Dysfunction

To determine the extent of CD-related toxicity and the subsequent amelioration of the toxicity with Vitamin C, E or Zinc co-administration, we measured AST, ALT, GGT, LDH activities in plasma. Our results demonstrated that enzyme activity levels were significantly (p<0.05) increased by 76.24% following CD treatment compared to 64.31%, 68.44% and 66.78% with pretreated Vitamin C, E and Zinc, respectively. However, the percent change was relatively low yet significant (p<0.05) recorded as 48.32, 41.05 and 28.03% with pre-treated Vitamin C, E and Zinc in combinations with CD groups, respectively (Table 2). But ALP activity was found to be significantly (p<0.05) decreased in all the experimental groups. This reduction suggests that CD induces liver toxicity and that Vitamin C, E and Zinc were able to protect against this toxicity. The Creatine, Glucose and Urea contents were found to be significantly elevated (p<0.05) in all the experimental groups compared to control group.

In the present study, an attempt was made to evaluate CD's toxic effects in rats; furthermore, we are very interested in knowing whether Vitamin C, E and Zinc as an antioxidant can recover the toxic effects caused by CD. CD has been recognized as one of the most toxic environmental and industrial pollutants and has been reported to induce oxidative damage. Elevated oxidative stress is due to the disruption of the prooxidant-antioxidant balance in the tissues. Earlier reports suggest that heavy metals manifest their toxic effects by enhanced production of reactive oxygen species (ROS) production, a major cellular source of oxidative stress.[1] ROS can damage every major cellular component, including membrane lipids, carbohydrates and deoxyribonucleic acid (DNA). The pathological consequence of such uncontrolled is wide spread tissue damage.[1] This present study's main objective was to evaluate the biochemical and pathological changes in following CD treatment in Wistar rats and the ability of Vitamin C, E, and Zinc to attenuate CD-mediated toxicity alone and in combination.

Table 1. Body and Relative Organ Weights of Control and Experimental Rats

Parameter	Control	Cadmium Treated	Cadmium+Vit-C Treated	Cadmium+Vit-E Treated	Cadmium+Zinc Treated	Cadmium+Vit-+Zinc Treated	Cadmium+Vit-E+Zinc Treated	Cadmium+Vit-E+C+Zinc Treated
Initial body weight (g)	225.28±10.04	226.13±10.59	225.11±9.14	224.19±9.74	225.82±8.54	225.32±8.49	226.18±8.44	225.79±8.48
Final body weight (g) PDC	256.56±10.95 +13.70[b]	193.18±10.32 +36.68[a]PDE	233.13±2.04 +3.56[c]+62.82[a]	238.35±10.14 +6.32[c]+66.47[a]	237.16±10.05 +5.02[c]+65.64[a]	242.11±10.13 +7.45[c]+69.09[a]	243.45±10.18 +7.64[c]+70.03[a]	246.77±10.73 +9.29[c]+72.35[a]
Body weight gain/ loss (g) in 45-days	31.28±1.15	32.95±1.14	8.02±0.33	14.16±0.48	11.84±0.56	16.79±0.64	17.27±0.84	20.98±0.88
Liver weight (g) PDC	8.95±0.18	5.73±0.15 -35.98[a]	7.36±0.16 -17.77[b]	7.39±0.17 -17.43[b]	7.42±0.17 -17.09[b]	8.45±0.18 -5.59[c]	8.42±0.23 -5.92[c]	8.18±0.21-8.61[c]
HSI of liver	3.49	2.97	3.16	3.10	3.13	3.49	3.46	3.31

All Values are Mean±SD of six individual observations. Values are statistically significant at (a) p<0.05, (b) p<0.01. (c) NS: Not Significant. PDC: Percent Deviation over control group. PDE: Percent Deviation over Experimental group i.e. Cadmium treated group. HIS: Histo Somatic Index.

Table 2. Changes in the Serum Enzymes and Blood Parameters in Control and Experimental Rats

Parameter/ Serum	Control	Cadmium Treated	Cadmium+Vit-C Treated	Cadmium+Vit-E Treated	Cadmium+Zinc Treated	Cadmium+Vit-C+Zinc Treated	Cadmium+Vit-E+Zinc Treated	Cadmium+Vit-E+C+Zinc Treated
AST1	48.77±1.52PDC	85.95±1.78 +76.24aPDE	80.13±1.82 +64.31a-6.77c	82.15±1.84 +68.44a-4.42c	81.34±1.79 +66.78a-5.36c	72.34±1.53 +48.32a-15.84b	68.79±1.74 +41.05a-19.97b	62.44±1.29 +28.03a-27.35a
ALT1	37.86±1.28PDC	67.94±1.29 +79.45aPDE	58.44±1.28 +54.36a-13.98b	58.79±1.62 +55.28a-13.47b	59.12±1.49 +56.15a-12.98b	54.71±1.62 +44.51a-19.77b	48.69±1.48 +28.61a-28.33a	45.73±1.54 +20.79a-32.69a
ALP1	102.39±3.78PDC	40.77±1.05 -60.18aPDE	61.11±1.22 -40.32a+49.89a	63.13±1.24 -38.34a+54.84a	64.31±1.39 -37.19a+57.74a	70.92±2.91 -30.74a+73.95a	75.99±2.88 -25.78a+86.39a	80.42±2.75 -21.46a+97.25a
GGT1	18.73±1.04PDC	30.76±1.34 +64.23aPDE	27.49±1.28 +46.77a-10.63b	26.89±1.48 +43.57a-12.58b	27.13±1.39 +44.85a-11.80b	25.14±1.12 +34.22a-18.27b	25.38±1.16 +35.51a-17.49b	22.15±1.19 +18.26a-27.99A
LDH1	214.73±10.05PDC	389.92±20.14 +81.59aPDE	340.15±15.94 +58.41a-12.76b	338.74±18.45 +57.75a-13.13b	342.05±20.10 +59.29a-12.28b	302.19±19.12 +40.73a-22.50a	290.13±18.12 +35.11a-25.59a	278.77±16.79 +29.82a-28.51a
Creatinine2	11.14±0.39PDC	17.34±0.48 +55.66aPDE	15.08±0.42 +35.37a-13.03b	15.14±0.41 +35.91a-12.69b	15.32±0.42 +37.52a-11.65b	13.79±0.44 +23.79a-20.47a	14.05±0.45 +26.12a-18.97a	13.34±0.42 +19.75a-23.07a
Glucose3	0.87±0.05PDC	1.73±0.12 +98.85aPDE	1.44±0.14 +65.52a-16.76b	1.38±0.13 +58.62a-20.23b	1.39±0.13 +59.77a-19.65b	1.22±0.14 +40.23a-29.48a	1.28±0.15 +47.13a-26.01a	1.13±0.14 +29.89a-34.68a
Urea2	0.53±0.03PDC	0.88±0.05 +66.04aPDE	0.74±0.05 +39.62a-15.91b	0.76±0.05 +43.40a-13.64b	0.78±0.06 +47.17a-11.36b	0.68±0.06 +28.30a-22.73a	0.69±0.08 +30.19a-21.59a	0.64±0.08 +20.75a-27.27a

All Values are Mean ±SD of six individual observations. Values are statistically significant at (a) p<0.05, (b) p<0.01. (c) NS: Not Significant.. PDC: Percent Deviation over control group. PDE: Percent Deviation over Experimental group, i.e. Cadmium treated group. AST: Aspertate Amino transferase; ALT: Alanine Amino transferase. ALP: Alkaline Phosphatase; GGT: Gamma-glutamyl transferase, LDH: Lactate dehydrogenase 1: IU/ml/hr; 2: g/lit; 3: mg/lit, 1,b Means in the same column not followed by the same letter differ significantly p<0.05

Body Weight and Relative Liver Weight

In toxicological studies, it has been reported that body and organ weights are considered as an important criterion for evaluating organ toxicity. The increase or decrease in body weights is a sign of toxic effects of xenobiotics. Body weight and relative liver weights of rats treated with CD or pretreated with Vitamins C, E or Zinc, either individually or in combination, followed by CD treatment, were significantly reduced. Immunization causes pain, distress, and inflammation, which reduces the animal's movement and appetite, thereby significantly decreasing food intake (anorexia or food avoidance) or poor food palatability due to CD treatment. CD-mediated toxicity involves the induction of oxidative stress resulting in alterations in the antioxidant status, leading to metabolic disorders and weight loss. Several authors reported that inflammation causes weight loss between 1-20% during CD exposure. Our study investigated Vitamin C, E, and Zinc's potential to attenuate CD-mediated toxicity and restore CD-treated rats' metabolic status, increasing food intake, body weight, and liver/body weight ratios. There was a significant (p<0.05) increase in body weight in the antioxidant-treated groups across all groups compared to the CD-treated animals.

Liver Dysfunction

From the results obtained, CD exposure clearly induces the damage that occurred in liver and other tissuesas observed through pathological studies. The liver and kidney are important organs for metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. The physiological function assigned to the liver and kidney suggests they are especially vulnerable to damage. As the liver is an important target organ for xenobiotic, we have also assessed the liver and its associated functions for CD-induced toxicity. Serum enzymes including ALP, ALT, AST, GGT and LDH are mainly considered as biomarkers for the evaluating of hepatic damage due to xenobiotic treatment. In the present study, following CD-exposure, serum AST, ALT, GGT and LDH were significantly elevated. In contrast, ALP activity showed a significant decrease in the activity levels than normal or control rats. In addition, increased levels of hepatic serum markers suggest an extensive liver injury. Lipid peroxidation is one of the main manifestations of oxidative damage, which always plays an important role in the toxicity of many xenobiotics.[2,20,21]

The data obtained in the present study also confirm that CD-intoxication causes a significant increase of lipid peroxidation concentration in liver tissue of rats. Since it causes lipid peroxidation in numerous tissues both *in vivo* and *in vitro*,[22] CD may induce oxidative stress by production of hydroxyl radicals,[23] superoxide anions, nitric oxide and hydrogen peroxide.[24,25] Cadmium exposure causes structural and functional damages to the cell membrane, significantly increasing permeability resulting in the leakage of hepatic enzymes into the blood. Furthermore, the liver damage due to oral administration of CD chloride was confirmed through the increase in the levels of plasma components, including bilirubin. Therefore, increased in the activities of AST and ALT activities in plasma is mainly attributed to the leakage of these enzymes from the liver cytosol into the blood stream. Reports are available that lysosomal instability caused by $CdCl_2$ resulted from leakage of hepatic enzymes including ALT, AST and ALP into the blood stream.[26] In the present study, a significant (p<0.05) increased AST activities; ALT changes may be attributed to the hepatic damage due to CD intoxication. Meanwhile, the alteration of serum ALP levels may also be attributed to cholestasis and acute hepatocellular necrosis. Several authors reported that due to CD treatment, the liver enzymes like SGOT, SGPT and ALP were significantly elevated compared to control group of rats, denoting liver dysfunction. Serum transaminases represented by AST and ALT were significantly elevated during CD-intoxication, indicating the loss of cellular integrity and the leakage of hepatic membrane. In the present study, the hepatocellular injury was associated with CD intoxication observed (Unpublished data).

El-Kady et al,[27] found that rats treated with CD (CD2+) ions alone showed a significant increase in serum enzymatic activities such as ALT and AST activities accompanied by significant decrease in the total protein content. Our results demonstrate that the marked changes in liver enzyme activities represent biomarkers for liver damage. Genchi et al[3] found that the increase in activities of these enzymes, including AST, ALT and ALP in serum after CD treatment, reflect the destructive effect of CD on cell membrane, resulting in increased release of functional enzymes from intracellular locations, which indicates the hepatotoxic effect of CD. Chavan et al[28] reported that serum GPT, ALP, bilirubin, blood urea were significantly increased in response to CD intoxication, indicating liver function impairment to an increase of CD-induced oxidative stress. Serum LDH activity also increased significantly due to the hepatocellular necrosis leading to leakage of enzyme into the blood stream. It has been previously reported that during liver damage, there was an observed decrease in anti-oxidant defenses in the liver.[3] The hepatic function test corroborated the histopathological lesions observed (Unpublished data). These observations indicated that marker enzymatic activity changes followed by the liver's overall histoarchitecture in response to CD-toxicity, which could be due to its toxic effects primarily by the generation of ROS causing a significant damage to the various membrane components of the cell. The prevention of lipid peroxidation is essential for all aerobic organisms so the organism is well-equipped with antioxidants that protect cells against the adverse effects of various toxicants.[29] Antioxidants' role in reversing the oxidative stress-induced due to xenobiotic intoxication has been of long-standing interest to basic scientists and clinicians.[29] The results obtained in the present study demonstrate that co-administration of Vitamin C, E and Zinc either individually or in combinations with CD reverted most of these altered biochemical parameter levels to within normal limits and substantially improved liver function. A partial amelioration of this damage by Vitamin C, E and Zinc would be attributed to antioxygenic role and Vitamin E as a free radical scavenger and an effective inhibitor of autocatalytic process of lipid peroxidation. Several authors reported that Vitamin E is the most important lipophilic antioxidant and exists mainly in the cellular membranes, thus helping to maintain the membrane stability.[9,30]

Cadmium exposure results in hyperglycemia and is due to the toxic action of this metal on the secretory activity of the pancreas. The elevation of serum glucose in the present study due to CD intoxication may be attributed to inhibition of insulin release from islets of langerhans.[31,32] Inhibition of glucose uptake in the target tissue or resistance to insulin action has also been reported.[33,34] Disruption in glucagon secretion results in high glycogen breakdown and a new supply of glucose production from other non-carbohydrate sources such as proteins.[35] However, there is an amelioration of blood glucose concentration in CD-treated animals with Vitamin C, E and Zinc, alone or in combination.

Consequently, treatment with Vitamin C, E and Zinc will improve glucose concentration in CD-treated Wistar rats. Improvement of the glucose status suggests that Vitamin C, E and Zinc supplementation will cause a decrease in CD effect in salt binding to biomolecules as well as improved insulin secretion by reducing glucose accumulation. Zinc protects enzymes, and ATP involved glucose metabolism. During tissue toxicity, including CD-induced toxicity, free radicals generated by oxidative damage to membrane lipids and lipoproteins can cause cellular damage and apoptosis.[20,37] Oxidative damage to cell membranes can further culminate into pathological changes in the histomorphology of exposed tissues. However, Vitamin C and E, natural antioxidants, can potently cause inhibition or scavenging of free radicals, thereby lessening the damage effects of tissue toxicants. It is an essential and Vitamin C and anti-toxin which potently functions to reduces the harmful effects of toxic agents in biological tissues. Each molecule of ascorbic acid contains two hydrogen atoms that bear two high-energy electrons, which can be readily donated to reduce oxidation by free radicals, thereby neutralizing or alleviating the harmful effects of tissue toxins[38]

Increased serum urea and creatinine levels in the CD-treated rat group may be attributed to an oxidative imbalance in the kidney, leading to elevated urea and creatinine in the blood and correlate with the earlier reports,[39] where urea level was elevated as a result of CD-intoxication. The significantly ($p<0.05$) elevated level of creatinine in the CD-intoxicated rat group may be attributed to the oxidative damage to the kidney. Deterioration of the kidney would permit creatinine release into the blood and agree with earlier reports, where CD administration leads to an elevated level of creatinine.[40] Kidney damage was also linked to the defect in infiltration. Shaffi[41] also reported that rise in creatinine level is an indication of renal-tubular damage due to CD-induced nephrotoxicity. The reduction of the urea and creatinine level by CD may suggest that Vitamin C, E and Zinc exerts hepato- and nephro protective effects when exposed to CD.

CONCLUSION

Based on the present study results, it could be evident that CD has a harmful and stressful effect on hepatic, renal and hematological tissues. However, either individually or in combinations Vitamin C, E and Zinc had protective effects against CD-induced oxidative damage or stress. Also, from our results, we conclude that Vitamin C, E and Zinc have potent antioxidant activity against CD toxicity. The consumption of foods rich in vitamin C & E is recommended to reduce the damage caused by CD's toxicity. Hence, Vitamin C, E and Zinc can be regarded as good therapeutic agents against CD toxicity.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Case Report

A Case Report of Severe Theophylline Poisoning: Management and Review of Literature

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ABSTRACT

Background

Theophylline poisoning leads to multisystem toxicity. Management of theophylline overdose is focused on stabilizing cardiovascular manifestations of arrhythmia and hypotension, correcting metabolic derangements, aborting seizures and removing the drug from the system. We present a case of refractory seizures and haemodynamic instability from theophylline poisoning and reviewed the literature to update the management of severe theophylline overdose.

Case Presentation

A 73-year-old Chinese gentleman presenting with chills and rigor was admitted for management of sepsis. While admitted suffered seizures which were refractory to benzodiazepine and anti-epileptic drugs. Based on his previous admission for theophylline overdose, serum levels were done confirming severe theophylline poisoning. He was resuscitated and subsequently started on haemodialysis following which seizures were eventually aborted when theophylline levels were successfully reduced.

Conclusion

Severe theophylline poisoning should be identified early and appropriate treatment initiated promptly. In the management of refractory hypotension, methylene blue and venoarterial-extracorporeal membrane oxygenation are reasonable rescue therapies to consider. Multi-dose activated charcoal and extracorporeal treatments for elimination of drugs should be administered in severe theophylline poisoning.

Keywords

Theophylline poisoning; Theophylline-associated seizures; Haemodialysis; Case report; Methylene blue; Venoarterial-extracorporeal membrane oxygenation; Multi-dose activated charcoal.

BACKGROUND

Theophylline is used to treat bronchospasm in asthma and chronic obstructive pulmonary disease (COPD) in adults. It blocks adenosine receptors and acts as a phosphodiesterase inhibitor, increasing beta-adrenergic effects and increases the release of catecholamines. Theophylline is a plant derived methyl xanthine compound that is similar to caffeine. It has up to 90% oral bioavailability and has a small volume of distribution of approximately 0.5 L/kg. It achieves peak serum concentration within 1 to 2-hours, although this is slowed in modified-release preparation. The half-life is approximately 8 to 11-hours with clearance lowered in overdose as elimination at high concentrations becomes zero-order.[1]

Acute theophylline toxicity manifestations can occur with doses from 7.5 mg/kg.[2] It presents with a constellation of clinical features that progress with severity as theophylline plasma concentration increases.[1] At lower toxic plasma levels, gastrointestinal features such as nausea and vomiting and neurological features such as headache, agitation and muscle tremor can occur.[3] With higher toxic plasma levels, more severe signs and symptoms such as hypotension, arrhythmia and seizures can occur. Metabolic derangements are more severe in acute toxicity and the ones commonly seen include hypokalaemia and hyperglycaemia.[4] In chronic theophylline intoxication, cardiovascular and neurological manifestation are more prominent. Risk of major toxicity resulting in morbidity correlates with serum theophylline levels in acute toxicity and extreme of ages in chronic toxicity.[5]

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The overall incidence of theophylline toxicity in Singapore is unknown. In America, the number of theophylline overdose reported to the American Association of Poison Control Centers (AAPCC) has decreased from 3100 cases reported in 1996[6] to 140 cases reported in 2019.[7] This is largely due to the decline in use of theophylline in the treatment of asthma and COPD with the emergence of other bronchodilator therapies. However, it still has a role in adult patients with asthma[8] and COPD as a third or fourth line therapy.[9]

The management of theophylline overdose is complex with life-threatening complications occurring across multiple systems in the body. The existing literature does cover the conventional management of theophylline overdose, however, there is a lack of information on further therapeutic measures that might need to be considered in the event of a severe theophylline overdose resulting in complications that are refractory to standard management. We aim to review the literature and provide an overview of the management of severe theophylline overdose.

CASE PRESENTATION

Our patient is a 73-year-old gentleman, with a past medical history of COPD, ischaemic cardiomyopathy, erosive esophagitis and seizure secondary to viral meningoencephalitis. He was brought into hospital *via* ambulance services with the presenting complain of chills and rigors of one day duration, with no recorded febrile temperature and occasional dry coughs for the past four-days. His wife provided further collaborative history and mentioned that he was alert, orientated and conversant during the episodes of rigors she had witnessed. The patient denied taking an overdose and his wife had not witnessed any attempt at poisoning. He arrived at the emergency department (ED) that evening with the following vitals: blood pressure (BP) of 83/54 mmHg, heart rate (HR) of 113 beats per minute, respiratory rate (RR) of 20 breaths per minute, oxygen saturation of 97% on room air. On examination, he was alert but noted to be breathless. There was scattered rhonchi heard bilaterally on examination of his lungs with no obvious crepitations. Notably, his right lower limb was swollen and red with pitting oedema. The rest of the physical examination was unremarkable. Electrocardiogram performed showed sinus tachycardia (HR 110, QTc 536 ms). Venous blood gas showed a pH of 7.4, serum bicarbonate of 19.8 mmol/L, normal anion gap acidosis with a corrected anion gap of 15, delta ratio 0.4 and lactate of 3.2 mmol/L. Renal and liver function tests were normal (serum potassium 4.2 mmol/L, serum glucose 8.2 mmol/L). Chest radiograph done to further evaluate his symptom of cough was unremarkable for congestion or consolidation. Serum theophylline, paracetamol and salicylate levels were sent in view of the recent history of theophylline poisoning.

His dyspnoea was initially attributed to COPD exacerbation and he was given intravenous (IV) hydrocortisone 100 mg and started on dry nebulisers. His hypotension and tachycardia on arrival was attributed to septic shock from a right lower limb cellulitis and he was started on IV Meropenem 1 g. After completing 500 mls of normal saline that was started by the paramedics, his blood pressure improved to 100/80 mmHg but he remained tachycardic at a PR of 130. Later that evening, he was noted to be increasingly dyspnoeic, with bibasal crepitations on lung examination and bilateral B lines seen on bedside ultrasound. There was now concern of a fluid overload state. Considering his dyspnoea and haemodynamic instability, he was admitted to a medical high dependency unit (HDU) for closer monitoring (Figure 1).

Figure 1. *Timeline Depicting the Main Progress During the First 7-Days of Admission*

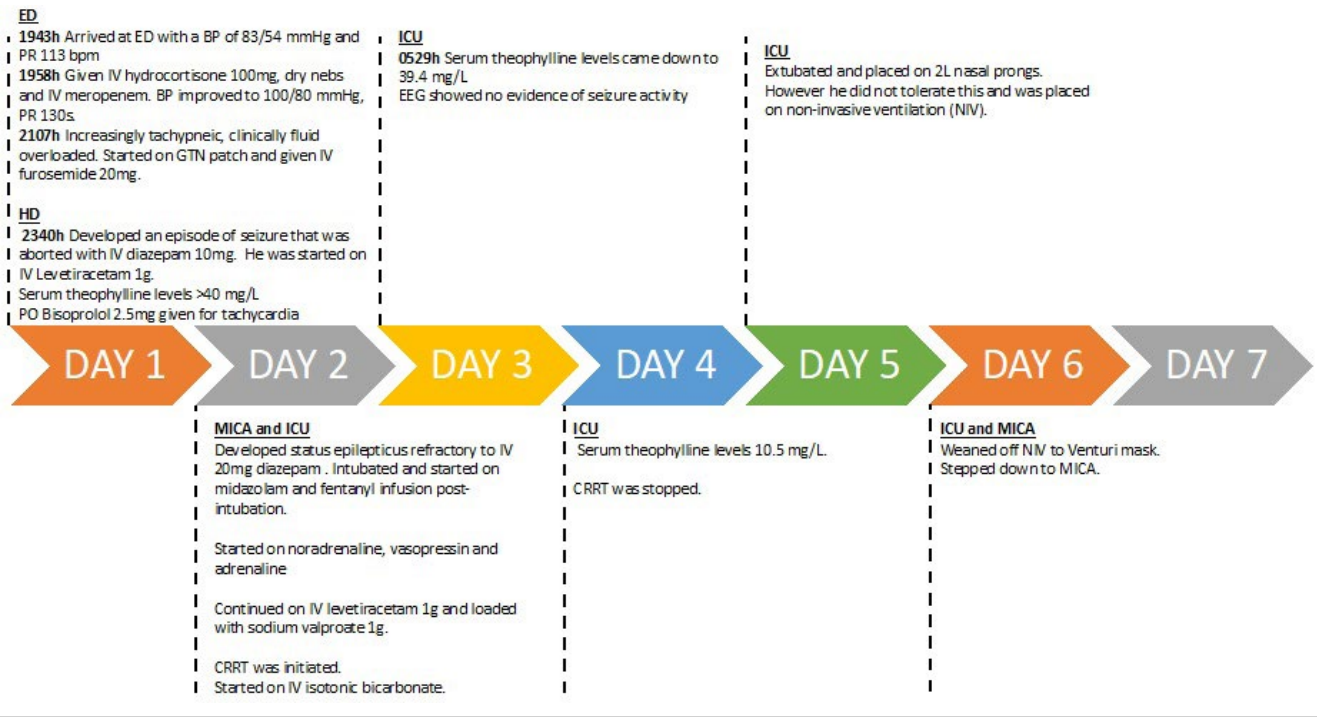
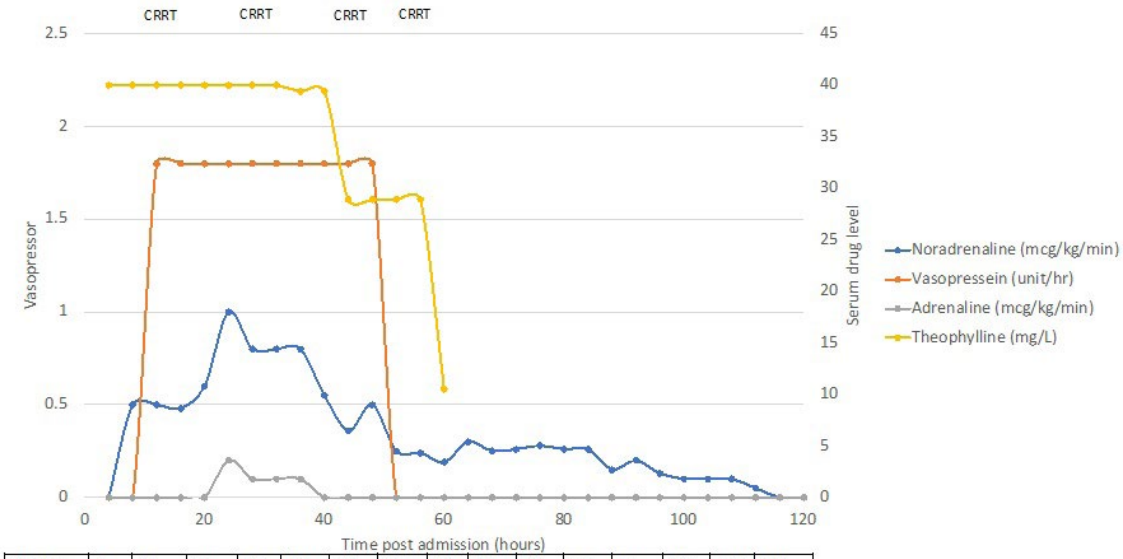


Figure 2. *Line Graph of Vasopressor Support and Serum Theophylline Levels Post Admission with CRRT as an Intervention*



Time (h)	8	16	24	32	40	48	56	64	72	80	88	96	104	112	120
BP (mmHg)	84/50	105/54	97/48	115/67	98/57	112/54	115/53	72/67	105/74	101/65	98/58	113/56	107/54	151/71	164/79
HR (bpm)	117	106	106	121	109	84	104	85	82	84	94	86	83	100	103

On arrival at the medical HDU he was noted to have seizure with right gaze preference, tonic-clonic jerking movements of his right upper limb, left upper limb and bilateral lower limbs stiffening. His seizure was aborted with IV diazepam 10 mg. He was loaded with IV levetiracetam 1 g. Serum theophylline levels were >40.0 mg/L (laboratory could not provide exact level) and salicylate and paracetamol levels not elevated. He was also given oral bisoprolol 2.5 mg for his sinus tachycardia (Figure 2).

In the early hours of the next day, he developed status epilepticus that was refractory to 2 doses of IV diazepam 10 mg. He was intubated for ongoing status epilepticus, a depressed GCS of E4V1M3 and haemodynamic instability. He was subsequently transferred to the intensive care unit (ICU). Post-intubation, he was started on midazolam and fentanyl infusion. He became haemodynamically unstable with hypotension post-intubation and was started on noradrenaline and vasopressin. He was reviewed by the neurologist and placed on regular IV levetiracetam 1 g twice daily and was loaded with a second anti-epileptic sodium valproate of 1 g and maintained on 400 mg twice daily after. He was reviewed by the renal physicians and continuous renal replacement therapy (CRRT) was initiated. He was also started on 500 mls of IV isotonic bicarbonate. Meropenem was stopped due to possible interactions with sodium valproate and his antibiotics was switched to piperacillin tazobactam. Later that evening, he was started on a third vasopressor adrenaline as blood pressure was persistently borderline.

On day 3 of admission, serum theophylline levels came down to 39.4 mg/L. He was weaned off adrenaline. Electroencephalography conducted later that day showed no evidence of seizure activity. Sodium valproate was held off and he was placed on levetiracetam 1 g every 8-hours due to deranged liver function tests.

On day 4 of admission, serum theophylline levels were now in the normal range of 10.5 mg/L and CRRT was stopped. He was subsequently extubated on day 5 and stepped down to medical HDU two days after. No further seizure episodes were observed during his hospital stay. An alert was raised in his electronic medical records to avoid further theophylline prescriptions. He was transferred to a subacute hospital for rehabilitations 55-days after admission with an outpatient neurology and respiratory follow-up.

DISCUSSION

Theophylline poisoning leads to multisystem dysfunction with high risk of serious cardiovascular instability and neurological complications. The immediate management of theophylline overdose is often that of its life-threatening complications.

Management of Theophylline Induced Cardiovascular Instability

Hypotension in theophylline overdose occurs as a result of beta 2 adrenergic stimulation and phosphodiesterase inhibition leading to vasodilation. Besides intravascular isotonic fluid boluses, vasoactive, predominately alpha adrenergic acts such as noradrenaline and phenylephrine[10] and beta adrenergic antagonists such as propranolol or esmolol[11] are used in theophylline induced hypotension. Common cardiac arrhythmias in theophylline poisoning, such as SVT, should also be addressed as they contribute to cardiovascular instability. Although adenosine is recommended by

advanced cardiac life support (ACLS) guidelines for treatment of supraventricular tachycardia (SVT), it has a high incidence of therapeutic failure when given in the setting of theophylline poisoning. This is the result of the short half-life of adenosine and the potent and profound adenosine receptor agonism of theophylline. A selective beta 1 antagonist such as esmolol is a good alternative as it can effectively terminate SVT and can be titrated to maintain this effect as a continuous infusion. It can be safely used in the setting of patients with asthma or heart failure.[12] If hypotension persists and is refractory to these standard treatment, other treatments can be explored. Vasopressin and its analogues can be considered an added on therapy to current vasoactive agents. It has been used in vasoplegic shock states in sepsis and after cardiac surgeries,[13] and has been used in drug overdosed states namely caffeine and calcium channel blockers[14,15] Methylene blue has also been used to treat vasoplegic shock[16] arising from a variety of settings such as septic shock,[17] cardiac surgery[18,19] and liver transplantation.[20] Data on the use of methylene blue in drug-induced vasoplegic shock have varied outcomes.[21] Further studies are required to determine the mortality benefit of using methylene blue in drug-induced vasoplegic shock. For now, methylene blue could be considered a form of rescue therapy in refractory vasoplegic shock that is unresponsive to conventional treatment. The last potential therapeutic option for refractory hypotension is the use of venoarterial-extracorporeal membrane oxygenation (VA-ECMO). VA-ECMO has been used in treatment of cardiogenic shock as a bridge to recovery of myocardium or until definitive treatment with heart transplant can be ascertained.[22] The use of VA-ECMO in drug-induced cardiogenic shock has been shown to improve haemodynamic and metabolic status in patients who do not respond to conventional medical treatment,[23] providing them the support they need until the toxic agent can be broken down or removed. VA-ECMO can be considered for use in drug-induced cardiogenic shock that is refractory to conventional therapy or in cardiac arrest.[24]

Management of Theophylline-Associated Seizures

Another serious complication of theophylline poisoning is theophylline-associated seizures (TAS), which can be intractable to first line seizure therapy with benzodiazepine, leading to status epilepticus.[25] Theophylline's antagonistic effect on adenosine receptor A2 and its inhibition of gamma-aminobutyric acid receptors are some of the proposed mechanism of theophylline's interaction with benzodiazepine, rendering it less useful in aborting TAS.[26] Hence, it is important to initiate early alternative therapy with anti-epileptic drugs (AED).[27] In the treatment of toxin-related seizures, pyridoxine[28] and propofol should be considered 2nd and 3rd line therapy after benzodiazepines.[29] There are however limited studies on the treatment of TAS specifically. In animal studies, the use of diazepam, clonazepam, phenobarbital or valproic acid increases threshold for theophylline-induced seizures[30] while phenobarbital is more effective than phenytoin in the termination of theophylline induced seizure.

Decontamination and Enhanced Elimination

Gastrointestinal decontamination with activated charcoal can be given if patients present early. In addition, the use of multi-dose activated charcoal (MDAC) can also be considered in patients who have ingested life-threatening amounts of theophylline.[31] Whole bowel irrigation might not be as effective in theophylline poisoning, as it reduces the capacity of charcoal to bind to theophylline. Recent clinical studies have substantiated the lack of improvement in the poisoned patient.[32] Extracorporeal treatments (ECTRs) enhance theophylline elimination with severe poisoning. It is indicated for use in acute overdose with theophylline serum levels >100 mg/L and in chronic overdose with serum theophylline level >60 mg/L or >50 mg/L if the patient is less than 6-months-old or more than 60-years-old. It is also indicated in presentations of severe toxicity, including seizures, life-threatening dysrhythmias, and shock. Rising serum theophylline levels despite optimal therapy should also prompt the use of ECTRs. As per the extracorporeal treatments in poisoning (EXTRIP) workgroup's recommendation, ECTRs should continue until clinical improvement or serum theophylline level is below 15 mg/L. The preferred form of ECTRs is intermittent hemodialysis, with hemoperfusion and CRRT being reasonable alternatives.[33] Exchange transfusion and peritoneal dialysis clearance rate seldom exceeded 15 ml/min which was deemed clinical significant only in newborns.[34]

CONCLUSION

In conclusion, severe theophylline poisoning should be identified early and appropriate treatment initiated promptly. In the management of refractory hypotension, methylene blue and VA-ECMO are reasonable rescue therapies to consider. MDAC and ECTRs should also be considered and administered in severe theophylline poisoning.

DECLARATIONS

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Written informed consent was obtained from the patient's legal guardian for publication of this case report and any accompanying images.

Availability of Data and Materials

Not applicable.

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Authors Contributions

ZJ and RP are major contributors in writing the manuscript. IH collated and summarised the information used in writing up the manuscript. All authors read and approved the final manuscript.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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