

Lipid emulsion attenuates theophylline-induced cardiotoxicity in rat cardiomyoblasts

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Abstract. Theophylline, a bronchodilator, induces cardiotoxicity due to its narrow therapeutic index. Lipid emulsion is used as a nonspecific antidote. However, its effects on theophylline-induced cardiotoxicity remain unknown. This study aimed to examine the effects of lipid emulsion on cardiotoxicity induced by toxic doses of theophylline in H9c2 rat cardiomyoblasts. The effects of theophylline and lipid emulsion (Lipofundin MCT/LCT), alone or in combination, on cell viability and migration were examined. Moreover, their effects on cleaved caspase-3, Bax/Bcl-XL ratio, and cleaved caspase-9 and caspase-8 expression, nuclear factor- κ B (NF- κ B) phosphorylation, and TUNEL-positive cells were examined. Theophylline treatment decreased cell viability. Lipofundin MCT/LCT (0.1–1%) with 50% long-chain and 50% medium-chain fatty acids inhibited the theophylline-induced reduction in cell viability (3×10^{-3} M). It partially inhibited the theophylline-induced decrease in cell migration. Further, it reversed the theophylline-induced increase in cleaved caspase-3, Bax/Bcl-XL ratio, and cleaved caspase-9 expression as well as NF- κ B (Ser536) phosphorylation. However, theophylline treatment did not alter cleaved caspase-8 expression. Additionally, lipid emulsion inhibited theophylline-induced increase in TUNEL-positive cells. Taken together, these results suggest that lipid emulsion reverses theophylline-induced apoptosis in cardiomyoblasts *via* inhibition of the mitochondrial apoptotic pathway associated with NF- κ B (Ser536) phosphorylation, indicating its potential for treating theophylline-induced cardiotoxicity.

Key words: Theophylline — Lipid emulsion — Cardiotoxicity — Apoptosis — Cell viability

Highlights

- Lipid emulsion, composed of 50% medium-chain and 50% long-chain fatty acid, inhibits theophylline-induced cardiotoxicity *via* blockade of the intrinsic apoptotic pathway associated with NF- κ B (Ser536) phosphorylation.

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Introduction

Bronchodilator theophylline, which inhibits phosphodiesterase and adenosine A₁ receptors, has adverse side effects, including cardiac arrhythmias, due to its narrow therapeutic index (Barnes 2010). Particularly, adenosine A₁ receptor inhibition, one of the mechanisms underlying theophylline-induced bronchodilation for bronchial asthma or chronic obstructive pulmonary disease, results in adverse effects, such as cardiac arrhythmia and seizure (Barnes 2010; Scurek and Brat 2024). A toxic dose of theophylline, which is plasma concentration-dependent (adverse effect-inducing plasma concentration of theophylline: 20 µg/mL; fatal plasma concentration of theophylline: 50 µg/mL), causes cardiac arrhythmia, including atrial fibrillation with rapid ventricular response, convulsion, and death (Schulz and Schmoldt 2003; Barnes 2010; Scurek and Brat 2024).

Currently, other bronchodilators are widely used to treat bronchial asthma and chronic obstructive pulmonary disease because of the severe adverse cardiac effects of theophylline (Journey and Bentley 2023). Moreover, the use of theophylline as an add-on therapy in severe asthma that is not well controlled by inhalation corticosteroids, and beta-2 agonist bronchodilators, and chronic obstructive pulmonary disease is controversial because of the serious adverse cardiac effects of theophylline (Barnes 2010; Scurek and Brat 2024). Thus, theophylline is primarily used to treat bradycardia and apnea in premature newborns in the United States (Journey and Bentley 2023). Nonetheless, theophylline toxicity is commonly observed in clinical practice (Chandrasekaran et al. 2020; Kong et al. 2021; Oxley-Oxland et al. 2022; Dinesh et al. 2025). Theophylline toxicity treatment involves gastrointestinal decontamination using activated charcoal or gastric lavage, intravenous fluid, phenylephrine, ondansetron for nausea and vomiting, cardiac arrhythmia treatment, benzodiazepine for seizure, and hemodialysis (Journey and Bentley 2023). Currently, lipid emulsion is routinely used to treat local anesthetic systemic toxicity (Lee and Sohn 2023). In addition, lipid emulsion has been reported to ameliorate cardiovascular depression induced by toxic doses of non-local anesthetic drugs, such as flecainide, amlodipine, bupropion, and quetiapine (Ok et al. 2020). Recently, lipid emulsion was reported to alleviate theophylline toxicity-induced hemodynamic depression, which is unresponsive to supportive treatment (Chandrasekaran et al. 2020). Further, theophylline enhances the apoptosis of eosinophils and lymphocytes by downregulating Bcl-2 expression, which is one of the underlying mechanisms by which theophylline exerts beneficial effects in allergic inflammation and asthma (Ohta and Yamashita 1999; Barnes 2010). Theophylline induces apoptosis in neutrophils *via* adenosine A_{2A} recep-

tor inhibition (Yasui et al. 2000) and in progenitor cells obtained from the blood of patients with asthma (Wang et al. 2003). In addition, lipid emulsion inhibits apoptosis induced by toxic doses of bupivacaine and propranolol in rat cardiomyoblasts, suggesting a lipid emulsion-mediated reduction in cardiotoxicity caused by these drugs (Ok et al. 2016, 2022). However, the effect of lipid emulsion on theophylline-induced cardiotoxicity remains unknown. This study aimed to examine the effect of lipid emulsion on cardiotoxicity caused by toxic doses of theophylline in H9c2 rat cardiomyoblasts and the underlying mechanism. Clarifying whether lipid emulsion can mitigate theophylline-induced cardiotoxicity may provide a novel therapeutic approach for treating life-threatening toxicity.

Materials and Methods

Materials

All reagents and compounds used in this study were of the highest commercially available purity. Theophylline and anti-β-actin antibody (#A5441) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Intralipid (20%) was sourced from Fresenius Kabi (Uppsala, Sweden), while Lipofundin MCT/LCT was provided by B. Braun Korea (Seoul, Korea). Antibodies specific to cleaved caspase-3 (#9661, 1:1000) and Bax (#2772, 1:2000) were acquired from Cell Signaling Technology (Beverly, MA, USA). Anti-Bcl-XL antibody (#sc-8392, 1:200) was sourced from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Cleaved caspase-8 antibody (#NB100-56116, 1:2000) was purchased from Novus Biologicals (Centennial, CO, USA), and cleaved caspase-9 antibody (#ADI-AAP-149-E, 1:1000) was obtained from Enzo Biochem Inc. (Farmingdale, NY, USA). Antibodies against NF-κB (#8242, 1:2000) and its phosphorylated form (Ser536) (#3033, 1:1000) were also supplied by Cell Signaling Technology.

Cell culture

H9c2 cardiomyoblasts derived from rat myocardium were obtained from the American Type Culture Collection (Rockville, MD, USA). These cells were propagated in Dulbecco's modified Eagle's medium (DMEM) with high glucose content (Gibco, Life Technologies, Grand Island, NY, USA) enriched with 10% fetal bovine serum and 1% penicillin-streptomycin solution (both from Gibco). Cell cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂, following the methodology outlined by Sim et al. (2025). Before exposure to the experimental compounds, the cells underwent a 16 h preincubation in DMEM devoid of serum.

Cell viability

Cell viability was assessed using Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan) in accordance with the manufacturer's protocol, as previously described by Sim et al. (2025). Briefly, H9c2 cells (2×10^4 cells/well) were plated in 24-well culture plates and exposed for 24 h to varying concentrations of theophylline, ranging from 3×10^{-4} M to 1×10^{-2} M. To investigate the potential protective effect of lipid emulsion – either Intralipid or Lipofundin MCT/LCT – against reduction in cell viability induced by a toxic dose of theophylline (3×10^{-3} M; > toxic plasma theophylline concentration of 20 $\mu\text{g/ml}$ or 1.1×10^{-4} M) (Schulz and Schmoldt 2003), cells were treated under different conditions: (1) 24 h exposure to 3×10^{-3} M theophylline alone; (2) pretreatment with lipid emulsion at concentrations of 0.1, 0.2, 0.5, or 1% for 1 h, followed by 24 h exposure to 3×10^{-3} M theophylline; or (3) exposure to lipid emulsion alone at the same concentrations for a total of 25 h. Following treatment, 200 μl of DMEM containing 10% CCK-8 reagent was added to each well, and the plates were incubated for 3 h at 37°C in a humidified incubator with 5% CO_2 . The absorbance was recorded at 450 nm using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). Cell viability was calculated using the following formula: Viability (%) = (absorbance of treated cells/absorbance of control cells) $\times 100$.

Wound-healing assay

To evaluate the effect of lipid emulsion on H9c2 cell migration following theophylline exposure, a wound-healing assay was conducted in accordance with the methodology outlined by Sim et al. (2025). H9c2 cells were seeded at a density of 3×10^5 cells/well in 6-well plates and incubated at 37°C in a humidified atmosphere containing 5% CO_2 . Upon reaching 90–100% confluency, a linear wound was created across the cell monolayer using a sterile 200 μl pipette tip. After washing the dish with phosphate-buffered saline to remove detached cells, fresh serum-free medium was added. Images of the wound area were captured using a Nikon Eclipse Ti2 microscope (Nikon, Tokyo, Japan). The cells were then assigned to the following treatment groups: (1) treatment with 3×10^{-3} M theophylline for 24 h; (2) preincubation with 0.5% Lipofundin MCT/LCT for 1 h followed by exposure to 3×10^{-3} M theophylline for 24 h; and (3) exposure to 0.5% Lipofundin MCT/LCT alone for 25 h. Post-treatment images were also acquired using the Nikon Eclipse Ti2 microscope. The extent of wound closure was quantified using the following equation: Wound healing (%) = (area of the wound 24 h post-scratch/initial wound area at 0 h) $\times 100$.

Western blot

Western blot analysis was employed to quantify the expression of cleaved caspase-3, cleaved caspase-9, cleaved caspase-8, Bax, and Bcl-XL, as well as to assess the phosphorylation levels of nuclear factor- κB (NF- κB) (Ser536) in H9c2 cells, based on the methodology outlined by Sim et al. (2025). To investigate cleaved caspase-3, and Bax/Bcl-XL ratio, cells were treated under the following conditions: 3×10^{-3} M theophylline for 12 h; 0.5% lipid emulsion (Lipofundin MCT/LCT) for 1 h followed by 12 h of 3×10^{-3} M theophylline; or 0.5% lipid emulsion alone for 13 h. To investigate cleaved caspase-9 expression, cells were treated under the following conditions: 3×10^{-3} M theophylline for 4 h; 0.5% lipid emulsion (Lipofundin MCT/LCT) for 1 h followed by 4 h of 3×10^{-3} M theophylline; or 0.5% lipid emulsion alone for 5 h. To analyze cleaved caspase-8 expression, cells received one of the following treatments: 3×10^{-3} M theophylline for 1 h; a 1 h preincubation with 0.5% lipid emulsion followed by 1 h of theophylline; or lipid emulsion alone for 2 h. The phosphorylation of NF- κB (Ser536) was examined in cells treated with 3×10^{-3} M theophylline for 12 h, pretreated with 0.5% lipid emulsion for 1 h followed by 12 h of theophylline exposure, or treated with 0.5% lipid emulsion alone for 13 h. Following all treatments, cells were lysed in radio-immunoprecipitation assay buffer (Cell Signaling Technology) containing protease and phosphatase inhibitors (Thermo Fisher Scientific, Rockford, IL, USA). Total protein concentration was quantified using a bicinchoninic acid assay kit (Thermo Fisher Scientific). Lysates were then denatured at 100°C for 10 min and subjected to 10–14% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by transfer to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with Tris-buffered saline with 0.5% Tween-20 (TBST) containing 5% skim milk or bovine serum albumin for 1 h at room temperature (22 – 27°C), then incubated overnight at 4°C with primary antibodies targeting cleaved caspase-3 (1:1000), cleaved caspase-8 (1:2000), Bax (1:2000), cleaved caspase-9 (1:1000), Bcl-XL (1:200), NF- κB (1:2000), or phospho-NF- κB (1:1000). After rinsing with TBST, the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG secondary antibodies (1:5000) for 1 h at room temperature. Detection of protein bands was carried out using the WesternBright™ ECL substrate (Advansta, Menlo Park, CA, USA), and signal acquisition was performed using the ChemiDoc™ Touch Imaging System (Bio-Rad Laboratories Inc., Berkeley, CA, USA). Densitometric analysis was conducted using ImageJ software (version 1.45s; NIH, Bethesda, MD, USA). Protein band intensities for cleaved caspase-3, cleaved caspase-8, cleaved caspase-9, Bax, and Bcl-XL were normalized against that for β -actin, which served as the loading control. For NF- κB (Ser536), phosphorylated protein

levels were normalized to total NF- κ B expression. All experiments were independently repeated four times.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

Apoptotic cells were assessed using the TUNEL assay following the protocol described by Sim et al. (2025). The assay was conducted using the In Situ Cell Death Detection Kit (Roche Applied Science, Indianapolis, IN, USA) in accordance with the manufacturer's guidelines. H9c2 cells were seeded onto glass coverslips placed in 6-well plates at a density of 1×10^5 cells/well and subjected to the following treatment regimens: exposure to 3×10^{-3} M theophylline for 12 h; pretreatment with 1% lipid emulsion (Lipofundin MCT/LCT) for 1 h followed by 12 h exposure to 3×10^{-3} M theophylline; or treatment with 1% lipid emulsion alone for 13 h. Following treatment, cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) to label nuclei. Fluorescence imaging was performed using a Nikon Eclipse Ti2 fluorescence microscope. The proportion of apoptotic cells was quantified using the following formula: Percentage of TUNEL-positive cells = (number of TUNEL-positive cells/number of DAPI-stained nuclei) $\times 100$.

Statistical analysis

The primary goal of this study was to examine the effect of lipid emulsion on theophylline-induced changes in cell viability in H9c2 rat cardiomyoblasts. Normality tests for all data were performed using the Kolmogorov-Smirnov test. The effects of theophylline and lipid emulsion (Lipofundin MCT/LCT or intralipid), alone or in combination, on cell viability and migration were analyzed using one-way analysis of variance (ANOVA), followed by Bonferroni's test. The effects of lipid emulsion and theophylline, alone or in combination, on cleaved caspase-3, -9, and -8 expression, Bax/

Bcl-XL ratio, NF- κ B phosphorylation, and TUNEL-positive cells were examined using one-way ANOVA, followed by Bonferroni's test. $p < 0.05$ was considered statistically significant.

Results

Effect of theophylline and lipid emulsion, alone or in combination, on cell viability

Toxic concentrations of theophylline (3×10^{-4} to 10^{-2} M) decreased the viability of H9c2 rat cardiomyoblasts ($p < 0.001$ vs. control, Fig. 1) (Schulz and Schmoldt 2003). One-way ANOVA showed a significant difference in cell viability among the groups ($F[5,90] = 2218$, $p < 0.001$), while Bonferroni's *post-hoc* test confirmed decreased cell viability under theophylline treatment ($T = 4.65, 21.54, 56.12, 70.35$, and 74.64 at 3×10^{-4} , 10^{-3} , 3×10^{-3} , 6×10^{-3} , and 10^{-2} M, respectively; degree of freedom [DF] = 30 at 3×10^{-4} to 10^{-2} M; $p < 0.001$ vs. control).

Lipofundin MCT/LCT (0.1–1%), which is composed of 50% long-chain and 50% medium-chain fatty acids, attenuated the decrease in cell viability caused by toxic concentrations of theophylline (3×10^{-3} M) ($p < 0.001$ vs. 3×10^{-3} M theophylline alone, Fig. 2A). Lipofundin MCT/LCT (0.1–0.5%) alone slightly increased cell viability (0.1%: $p < 0.01$ vs. control; 0.2 and 0.5%: $p < 0.001$ vs. control; Fig. 2A). One-way ANOVA showed a significant difference in cell viability among the groups ($F[9,150] = 1521$, $p < 0.001$). Bonferroni's *post-hoc* test confirmed that theophylline (3×10^{-3} M) decreased cell viability compared with the control ($T = 56.43$, DF = 30, $p < 0.001$) and that lipid emulsion (Lipofundin MCT/LCT) plus theophylline increased cell viability compared with theophylline alone ($T = 4.42, 8.07, 13.35$, and 13.52 at 0.1, 0.2, 0.5, and 1% lipid emulsion, respectively; DF = 30 at 0.1–1% lipid emulsion,

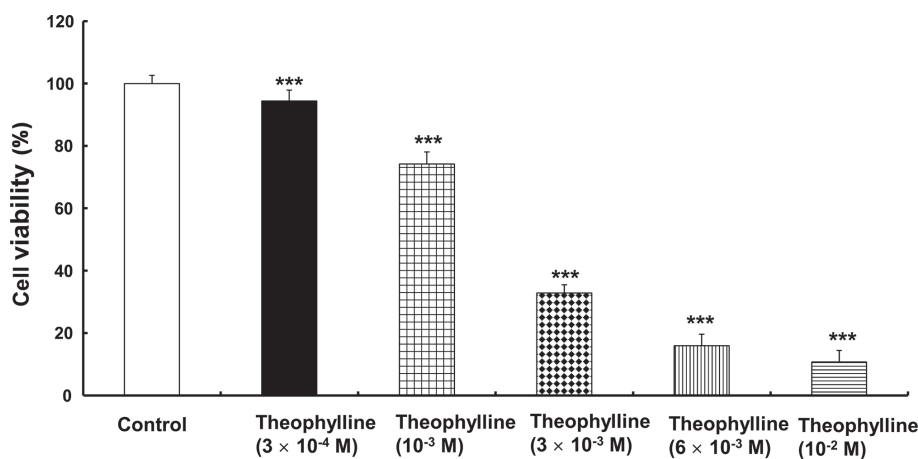


Figure 1. Effect of theophylline on H9c2 rat cardiomyoblast viability. Data ($n = 4$) are shown as mean $\pm$ SD. n indicates the number of independent experiments. *** $p < 0.001$ vs. Control.

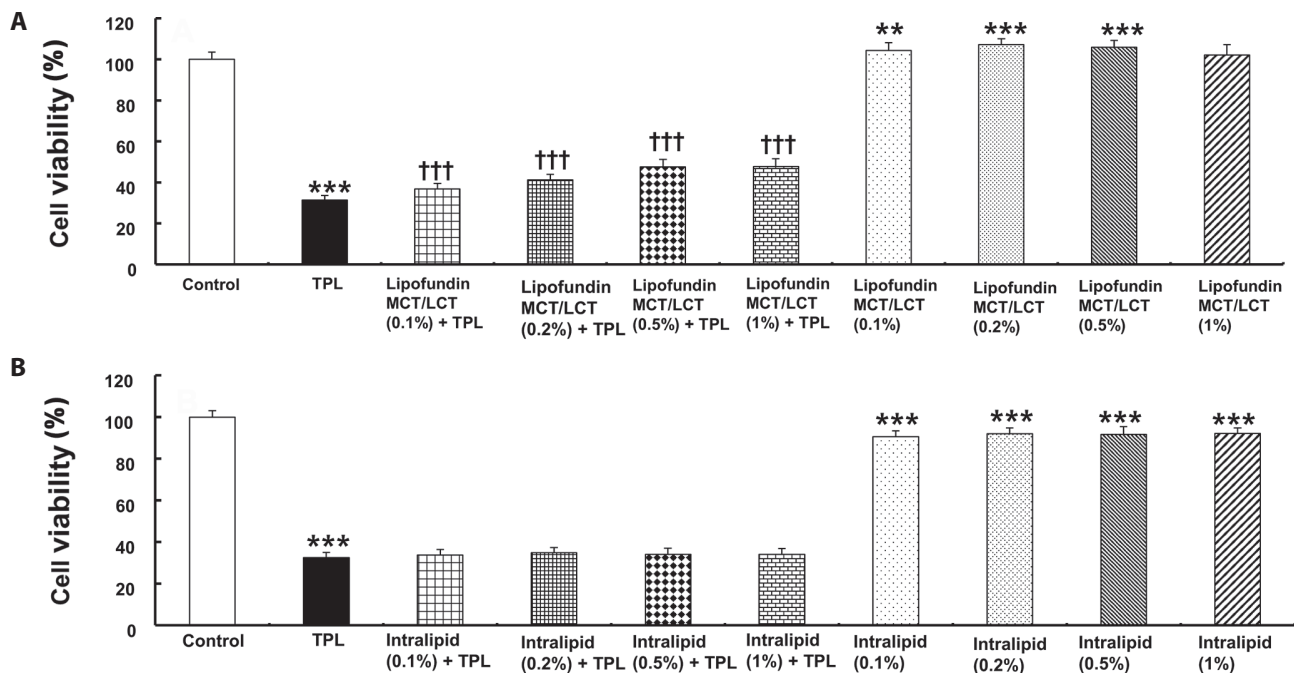


Figure 2. Effect of lipid emulsions Lipofundin MCT/LCT (A) and Intralipid (B) on 3×10^{-3} M TPL-induced decrease in cell viability of H9c2 rat cardiomyoblasts. Data ($n = 4$) are shown as mean $\pm$ SD. n indicates the number of independent experiments. ** $p < 0.01$ and *** $p < 0.001$ vs. Control. ††† $p < 0.001$ vs. TPL alone.

$p < 0.001$). In addition, Bonferroni's post-hoc test confirmed that Lipofundin MCT/LCT (0.1–0.5%) alone increased cell viability ($T = 3.507, 5.824$, and 4.804 at 0.1, 0.2, and 0.5% lipid emulsion, respectively; $DF = 30$ at 0.1–0.5% lipid emulsion; 0.1% lipid emulsion: $p < 0.01$ vs. control; 0.2 and 0.5% lipid emulsion: $p < 0.001$ vs. control). However, Intralipid, which is composed of 100% long-chain fatty acids, had no effect on theophylline toxic dose (3×10^{-3} M)-induced decrease in cell viability (Fig. 2B). In contrast, Intralipid (0.1–1%) alone slightly decreased cell viability ($p < 0.001$ vs. control, Fig. 2B). One-way ANOVA showed a significant difference in cell viability among the groups ($F[9,150] = 1925$, $p < 0.001$). Bonferroni's post-hoc test confirmed that Intralipid (0.1–1%) alone decreased cell viability ($T = 9.365, 7.954, 8.175$, and 7.817 at 0.1, 0.2, 0.5, and 1% lipid emulsion, respectively; $DF = 30$ at 0.1–1% lipid emulsion; $p < 0.001$ vs. control).

Effect of theophylline and lipid emulsion, alone or in combination, on cell migration

Theophylline (3×10^{-3} M) inhibited cell migration ($p < 0.001$ vs. the control; Fig. 3). However, lipid emulsion (0.5% Lipofundin MCT/LCT) partially reversed the decrease in cell migration caused by theophylline (3×10^{-3} M) ($p < 0.001$ vs. 3×10^{-3} M theophylline, Fig. 3). One-way ANOVA showed a significant difference in cell migration among the four

groups ($F[3,28] = 530.4$, $p < 0.001$). Bonferroni's post-hoc test confirmed decreased cell migration under theophylline treatment ($T = 31.62$, $DF = 14$, $p < 0.001$ vs. control) and increased cell migration under lipid emulsion (Lipofundin MCT/LCT) plus theophylline treatment ($T = 9.578$, $DF = 14$, $p < 0.001$ vs. theophylline alone).

Effect of theophylline and lipid emulsion, alone or in combination, on cleaved caspase-3, Bax/Bcl-XL ratio, cleaved caspase-9, and caspase-8 expression, and NF- κ B phosphorylation

Theophylline (3×10^{-3} M) increased cleaved caspase-3 expression ($p < 0.001$ vs. control, Fig. 4A). However, the lipid emulsion (0.5%, Lipofundin MCT/LCT) attenuated theophylline (3×10^{-3} M)-induced cleaved caspase-3 expression ($p < 0.001$ vs. theophylline, Fig. 4A). One-way ANOVA showed a significant difference in cleaved caspase-3 expression among the four groups ($F[3,20] = 3131$, $p < 0.001$), with Bonferroni's post-hoc test confirming increased cleaved caspase-3 expression under theophylline treatment ($T = 68.06$, $DF = 10$, $p < 0.001$ vs. control) and decreased cleaved caspase-3 expression under lipid emulsion (Lipofundin MCT/LCT) plus theophylline treatment ($T = 81.41$, $DF = 10$, $p < 0.001$ vs. theophylline alone). In addition, theophylline (3×10^{-3} M) increased Bax/Bcl-XL ratio ($p < 0.001$ vs. control, Fig. 4B), while the lipid emulsion (0.5% Lipofun-

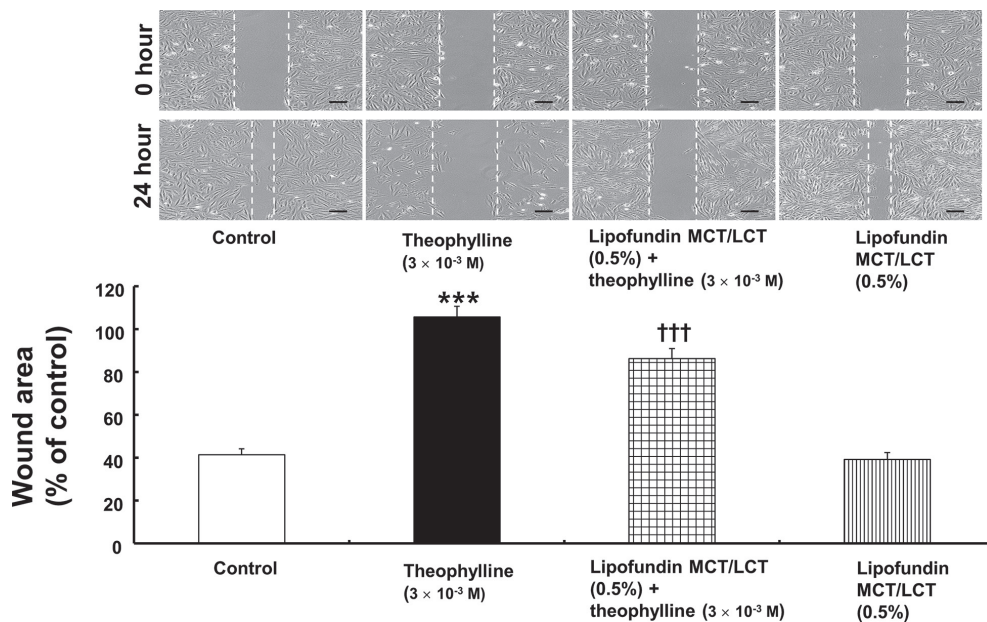


Figure 3. Effect of theophylline (3×10^{-3} M TPL) and lipid emulsion (0.5%, Lipofundin MCT/LCT), alone or in combination, on cell migration in H9c2 rat cardiomyoblasts. Data ($n = 4$) are shown as mean $\pm$ SD. n indicates the number of independent experiments. Scale bar: 100 μ m. *** $p < 0.001$ vs. control. ††† $p < 0.001$ vs. theophylline.

din MCT/LCT) attenuated the augmented ratio of Bax/Bcl-XL induced by theophylline (3×10^{-3} M) ($p < 0.001$ vs. 3×10^{-3} M theophylline alone, Fig. 4B). One-way ANOVA revealed a significant difference in Bax/Bcl-XL ratio among the four groups ($F[3,20] = 33.64$, $p < 0.001$), with Bonferroni's post-hoc test confirming increased Bax/Bcl-XL ratio under theophylline treatment ($T = 7.585$, $DF = 10$, $p < 0.001$ vs. control) and decreased Bax/Bcl-XL ratio under lipid emulsion (Lipofundin MCT/LCT) plus theophylline treatment ($T = 6.551$, $DF = 10$, $p < 0.001$ vs. theophylline alone). Theophylline (3×10^{-3} M) augmented cleaved caspase-9 expression ($p < 0.001$ vs. control, Fig. 4C), while the lipid emulsion (0.5%

Lipofundin MCT/LCT) reversed the theophylline toxic dose (3×10^{-3} M)-induced cleaved caspase-9 expression ($p < 0.001$ vs. 3×10^{-3} M theophylline, Fig. 4C). One-way ANOVA showed a significant difference in cleaved caspase-9 expression among the four groups ($F[3,24] = 48.58$, $p < 0.001$), with Bonferroni's post-hoc test confirming increased cleaved caspase-9 expression under theophylline treatment ($T = 9.273$, $DF = 12$, $p < 0.001$ vs. control) and decreased cleaved caspase-9 expression under lipid emulsion (Lipofundin MCT/LCT) plus theophylline treatment ($T = 6.059$, $DF = 12$, $p < 0.001$ vs. theophylline alone). However, theophylline (3×10^{-3} M), alone or in combination with lipid emulsion

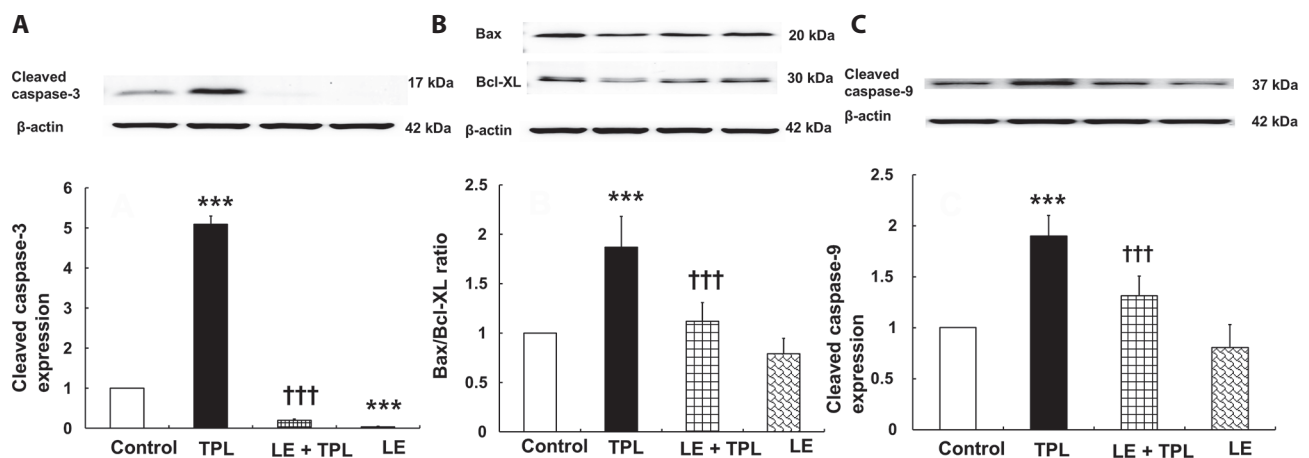


Figure 4. Effect of theophylline (3×10^{-3} M TPL) and lipid emulsion (LE, Lipofundin MCT/LCT, 0.5%), alone or in combination, on cleaved caspase-3 expression (A), Bax/Bcl-XL ratio (B), and cleaved caspase-9 expression (C) in H9c2 rat cardiomyoblasts. Data ($n = 4$) are shown as mean $\pm$ SD. n indicates the number of independent experiments. *** $p < 0.001$ vs. Control. ††† $p < 0.001$ vs. TPL alone.

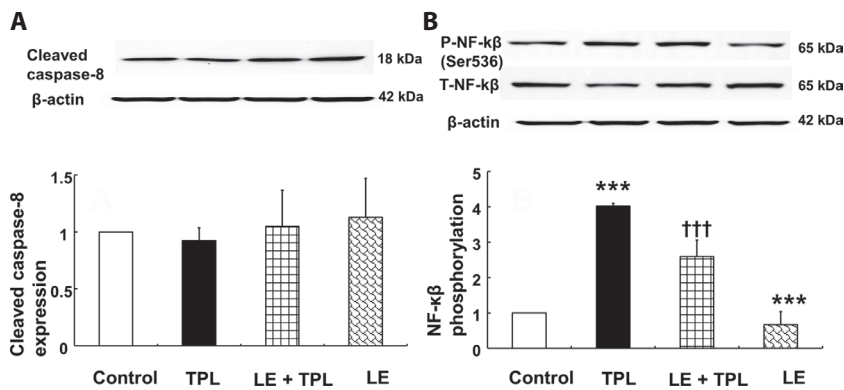


Figure 5. Effect of theophylline (3×10^{-3} M TPL) and lipid emulsion (LE, Lipofundin MCT/LCT, 0.5%), alone or in combination, on cleaved-8 expression (A), and nuclear factor- κ B (NF- κ B) (Ser532) phosphorylation (B) in H9c2 rat cardiomyoblasts. Data ($n = 4$) are shown as mean $\pm$ SD. n indicates the number of independent experiments. *** $p < 0.001$ vs. Control. ††† $p < 0.001$ vs. TPL. P-NF- κ B (Ser532), phosphorylated NF- κ B (Ser532); T-NF- κ B, total NF- κ B.

(0.5%, Lipofundin MCT/LCT), did not significantly alter cleaved caspase-8 expression (Fig. 5A). One-way ANOVA showed no significant difference in cleaved caspase-8 expression among the four groups ($F[3,20] = 0.7755$, $p = 0.5213$). Further, theophylline (3×10^{-3} M) increased NF- κ B (Ser532) phosphorylation ($p < 0.001$ vs. control, Fig. 5B). In contrast, lipid emulsion (0.5%, Lipofundin MCT/LCT) reversed the theophylline-induced NF- κ B (Ser532) phosphorylation (3×10^{-3} M) ($p < 0.001$ vs. theophylline alone, Fig. 5B). One-way ANOVA showed a significant difference in NF- κ B (Ser532) phosphorylation among the four groups ($F[3,24] = 186.6$, $p < 0.001$), with Bonferroni's *post-hoc* test confirming increased NF- κ B (Ser532) phosphorylation under theophylline treatment ($T = 18.85$, $DF = 12$, $p < 0.001$ vs. control) and decreased NF- κ B (Ser532) phosphorylation under lipid

emulsion (Lipofundin MCT/LCT) plus theophylline treatment ($T = 8.929$, $DF = 12$, $p < 0.001$ vs. theophylline alone).

Effect of theophylline and lipid emulsion, alone or in combination, on TUNEL-positive cells

Theophylline (3×10^{-3} M) increased the number of TUNEL-positive cells ($p < 0.001$ vs. the control, Fig. 6). However, the lipid emulsion (1% Lipofundin MCT/LCT) attenuated the augmented number of TUNEL-positive cells induced by theophylline (3×10^{-3} M) ($p < 0.001$ vs. 3×10^{-3} M theophylline, Fig. 6). One-way ANOVA showed a significant difference in TUNEL-positive cells among the four groups ($F[3,16] = 18.46$, $p < 0.001$), with Bonferroni's *post-hoc* test confirming increased number of TUNEL-positive cells

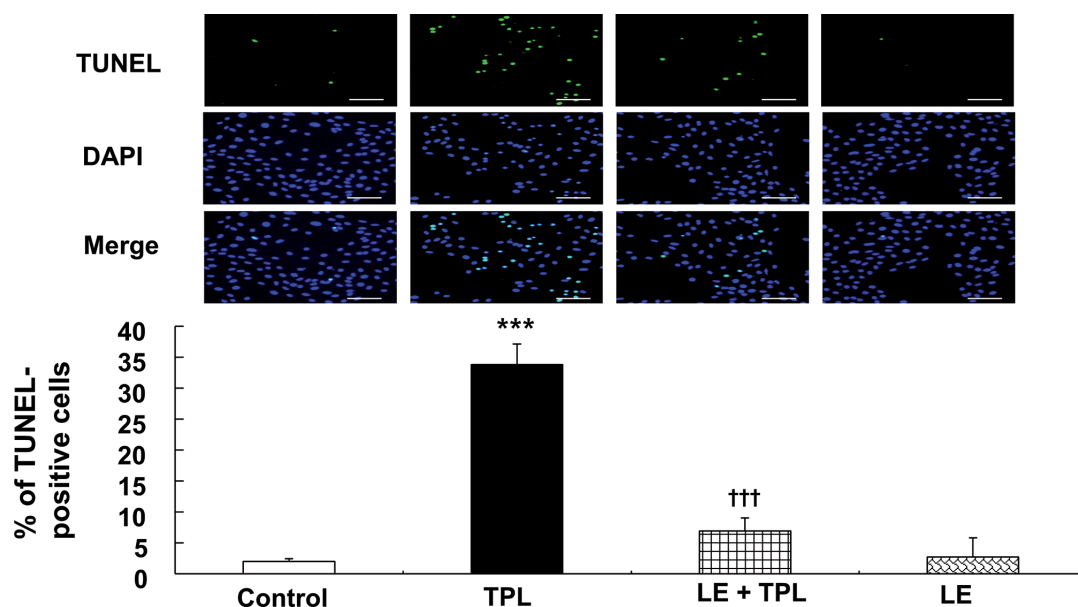


Figure 6. Effect of theophylline (3×10^{-3} M TPL) and lipid emulsion (LE, Lipofundin MCT/LCT, 1%), alone or in combination, on TUNEL-positive cells in H9c2 rat cardiomyoblasts. Data ($n = 5$) are shown as mean $\pm$ SD. n indicates the number of independent experiments. Scale bar: 100 μ m. *** $p < 0.001$ vs. Control. ††† $p < 0.001$ vs. TPL.

under theophylline treatment ($T = 20.22$, $DF = 8$, $p < 0.001$ vs. control) and decreased number of TUNEL-positive cells under lipid emulsion (Lipofundin MCT/LCT) plus theophylline treatment ($T = 18.80$, $DF = 8$, $p < 0.001$ vs. theophylline alone).

Discussion

This study suggests that lipid emulsion, which is composed of 50% medium-chain and 50% long-chain fatty acids, inhibits theophylline-induced cardiotoxicity *via* blockade of the intrinsic apoptotic pathway associated with NF- κ B (Ser532) phosphorylation. The main findings of this study are as follows: 1) Lipofundin MCT/LCT reversed theophylline toxic dose-induced decrease in cell viability, while Intralipid had no such effect. 2) Lipid emulsion inhibited theophylline-induced increase in cleaved caspase-3 and Bax/Bcl-XL ratio. 3) Lipid emulsion reversed theophylline-induced increase in the number of TUNEL-positive cells.

Lipid emulsion reversed decreased cell viability induced by bupivacaine and propranolol (Ok et al. 2016, 2022), which may contribute to inhibition of cardiotoxicity. Theophylline inhibits interleukin-5-induced enhanced cell viability in eosinophils (Chung et al. 2000). Further, theophylline (10^{-4} – 10^{-3} M) inhibits the survival of eosinophils pretreated with interleukin-5 (10 ng/ml) (Ohta et al. 1996). Similar to previous reports (Ohta et al. 1996; Chung et al. 2000), theophylline (3×10^{-4} to 10^{-2} M) decreased the viability of H9c2 rat cardiomyoblasts in a concentration-dependent manner. Lipofundin MCT/LCT (0.1 to 1%) reversed theophylline toxic dose (3×10^{-3} M)-induced reduction in cell viability, while Intralipid (0.1–1%), which is composed of 100% long-chain fatty acid, had no such effect (Wanten and Calder 2007). Moreover, Lipofundin MCT/LCT (0.1–0.5%) alone slightly increased cell viability, while intralipid (0.1–1%) alone slightly reduced cell viability (Fig. 2). This differential effect may influence recovery from the reduction in cell viability induced by theophylline toxicity. Taken together, these results suggest that medium-chain fatty acids may contribute to the reversal of the decreased cell viability caused by toxic doses of theophylline. Further studies are needed to examine the type of medium-chain fatty acid primarily involved in this reversal. In addition to reversing the theophylline-induced decrease in cell viability, lipid emulsion (Lipofundin MCT/LCT) also partially reversed the reduction in cell migration induced by toxic doses of theophylline (3×10^{-3} M) (Fig. 3).

Apoptosis proceeds *via* two principal signaling pathways: intrinsic and extrinsic pathways (MacFarlane 2009). The intrinsic pathway, triggered by mitochondrial dysfunction, involves the release of cytochrome c from the mitochondrial intermembrane space (MacFarlane 2009). This process is tightly regulated by pro-apoptotic proteins, such as Bax and

Bak, as well as anti-apoptotic members of the Bcl-2 family, including Bcl-2 and Bcl-XL, ultimately culminating in the activation of cleaved caspase-9 (MacFarlane 2009). In contrast, the extrinsic pathway is initiated by death receptor stimulation, which activates cleaved caspase-8 (MacFarlane 2009). Both apoptotic cascades converge upon activation of cleaved caspase-3, a key executioner enzyme, resulting in programmed cell death (MacFarlane 2009). Herein, theophylline (3×10^{-3} M) increased cleaved caspase-3 expression, but the lipid emulsion (Lipofundin MCT/LCT, 0.5%) reduced the theophylline-induced increase in cleaved caspase-3 expression. Bcl-2 expression, which is increased by interleukin-5 in eosinophils, is inhibited by theophylline (Chung et al. 2000). Theophylline induces apoptosis by decreasing Bcl-2 expression in eosinophils pretreated with interleukin-5, and in lymphocytes, which is mediated by increased cyclic adenosine monophosphate formation due to inhibition of phosphodiesterase (Ohta and Yamashita 1999). Theophylline-induced adenosine A_{2A} receptor inhibition induces apoptosis in neutrophils (Yasui et al. 2000). Moreover, theophylline induces apoptosis by downregulating Bcl-2 in blood progenitor cells of patients with asthma (Wang et al. 2003). In addition, a theophylline derivative induced apoptosis with decreased Bcl-XL expression in a breast cancer cell line (MDA-MB-231 cell) (Afifi et al. 2025). Furthermore, fenethylline, a mixture of theophylline and amphetamine, induces cardiac toxicity and myocardial apoptosis by increasing caspase-3 and Bax expression and decreasing Bcl-2 expression (Shehata et al. 2025). Similar to these previous reports (Ohta and Yamashita 1999; Chung et al. 2000; Yasui et al. 2000; Wang et al. 2003; Afifi et al. 2025; Shehata et al. 2025), our study indicated that theophylline increased Bax/Bcl-XL ratio, but did not significantly affect cleaved caspase-8 expression. However, lipid emulsion (0.5%) reversed the increase in Bax/Bcl-XL ratio induced by theophylline (3×10^{-3} M). Taken together, these results suggest that lipid emulsion inhibits theophylline-induced apoptosis by inhibiting the mitochondrial apoptotic pathway. In agreement with this result, lipid emulsion attenuated the theophylline-induced increase in the number of TUNEL-positive cells. NF- κ B is generally associated with anti-apoptosis (Khandelwal et al. 2011). However, cellular stresses, such as hypoxia, and DNA-damaging agents induce nuclear translocation of the RelA/NF- κ B complex and promote apoptosis (Khandelwal et al. 2011). Additionally, fenethylline-induced cardiotoxicity involves increased NF- κ B gene expression (Shehata et al. 2025). Considering these reports (Khandelwal et al. 2011; Shehata et al. 2025), our results showing that lipid emulsion prevented the theophylline-induced increase in NF- κ B (Ser532) phosphorylation suggest that lipid emulsion may inhibit theophylline-induced apoptosis by suppressing the NF- κ B-mediated apoptotic pathway. Further studies are

needed to examine potential upstream cellular signaling pathways – including the cyclic adenosine monophosphate accumulation, adenosine receptor inhibition, and NF- κ B-related pathways – that initiate the theophylline-induced intrinsic apoptotic pathway. This will help elucidate the detailed cellular signaling mechanisms associated with theophylline toxic dose-induced apoptosis.

Recently, lipid emulsion has been reported to be effective in ameliorating cardiovascular depression (distributive shock) caused by theophylline toxicity, which is unresponsive to supportive treatments, including intravenous fluid, vasopressors, calcium gluconate, and KCl (Chandrasekara et al. 2020). In addition, lipid emulsion reportedly alleviates cardiovascular collapse caused by toxic doses of non-local anesthetic drugs with high lipid solubility (log P [octanol/water partition coefficient] > 2), including flecainide (log P: 3.8), quetiapine (log P: 2.81), bupropion (log P: 3.2), and amlodipine (log P: 3) (Ok et al. 2020). The therapeutic action of lipid emulsion against drug toxicity involves both indirect and direct mechanisms (Lee and Sohn 2023). The predominant indirect mechanism, often referred to as the “lipid shuttle,” proposes that lipid emulsion sequesters lipophilic agents – such as bupivacaine (log P = 3.41) – from highly perfused tissues like the heart and central nervous system (Lee and Sohn 2023). Following sequestration, drug-laden lipid particles are redistributed to peripheral sites, including the liver, skeletal muscle, and adipose tissue, where they are detoxified and stored (Lee and Sohn 2023). In parallel, lipid emulsion exerts several direct pharmacological effects, including enhancement of myocardial contractility, provision of free fatty acids as metabolic substrates, mitigation of mitochondrial dysfunction, and suppression of nitric oxide synthesis (Lee and Sohn 2023). Considering these underlying mechanisms (Lee and Sohn 2023), in contrast to lipid soluble drugs like bupivacaine (log P: 3.41), theophylline (log P: –0.02) is not lipid soluble. Thus, the lipid emulsion-mediated recovery of the distributive shock caused by theophylline toxicity observed in previous case reports may be due to inhibition of nitric oxide release by lipid emulsion (Chandrasekara et al. 2020; Lee and Sohn 2023). In other words, toxic doses of theophylline cause severe endothelial nitric oxide-dependent vasodilation in isolated endothelium-intact rat aortas, which may lead to severe hypotension due to inhibition of nitric oxide degradation induced by phosphodiesterase inhibition (Murad 2006; Park et al. 2023).

This study has some limitations. First, an *in vitro* approach was used, although an *in vivo* study is more appropriate for examining the effect of lipid emulsion on hemodynamic depression caused by theophylline toxicity. Second, the H9c2 rat cardiomyoblast cell line was used. However, cultured primary cardiomyocytes are more appropriate, because cell lines may have been modified from the original cells (Kaur and Dufour 2012). Despite these limitations, our results sug-

gest that lipid emulsion may contribute to the attenuation of theophylline-induced cardiotoxicity.

Collectively, these results suggest that lipid emulsion reverses apoptotic cell death of cardiomyoblasts caused by toxic doses of theophylline *via* inhibition of the intrinsic apoptotic pathway associated with NF- κ B (Ser532) phosphorylation. This effect may contribute to the reduction in the cardiotoxicity induced by toxic doses of theophylline. However, further *in vivo* studies to examine the effect of lipid emulsion on cardiac toxicity caused by theophylline are needed to confirm the results of this *in vitro* study.

Conflict of interest. The authors declare no conflict of interest.

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References

- Afifi OS, Abdellatef AA, Abd El Razik HA, Shams El-Dine SEA, El-Tombary AA, Elrewiny MA, Hayakawa Y, El-Yazbi AF, Nematalla HA, Elzahhar PA (2025): Revisiting multi-pathway modulation with theophylline derivatives: Targeting STAT3, NF- κ B, and apoptotic signaling in breast cancer. *Bioorg. Chem.* **163**, 108708 <https://doi.org/10.1016/j.bioorg.2025.108708>
- Barnes PJ (2010): Theophylline. *Pharmaceuticals (Basel)* **18**, 725–747 <https://doi.org/10.3390/ph3030725>
- Chandrasekaran VP, Parthasarathy A, Muruga B, Senthamil, Tharanesh (2020): Theophylline toxicity: Successful management of a patient with distributive shock secondary to drug overdose. *Am. J. Emerg. Med.* **38**, 2491.e1–2491.e2 <https://doi.org/10.1016/j.ajem.2020.05.112>
- Chung IY, Nam-Kung EK, Lee NM, Chang HS, Kim DJ, Kim YH, Park CS (2000): The downregulation of Bcl-2 expression is necessary for theophylline-induced apoptosis of eosinophil. *Cell. Immunol.* **203**, 95–102 <https://doi.org/10.1006/cimm.2000.1678>
- Dinesh V, Varshini DR, Mohanan AA, Amrithanand VT (2025): Navigating the storm: A case of severe theophylline toxicity and its management. *Medical Reports* **12**, 100191 <https://doi.org/10.1016/j.hmedic.2025.100191>
- Journey JD, Bentley TP (2026): Theophylline Toxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing
- Kaur G, Dufour JM (2012): Cell lines: Valuable tools or useless artifacts. *Spermatogenesis* **2**, 1–5 <https://doi.org/10.4161/spmg.19885>
- Khandelwal N, Simpson J, Taylor G, Rafique S, Whitehouse A, Hiscox J, Stark LA (2011): Nucleolar NF- κ B/RelA mediates

- apoptosis by causing cytoplasmic relocation of nucleophosmin. *Cell. Death. Differ.* **18**, 1889-1903
<https://doi.org/10.1038/cdd.2011.79>
- Kong A, Ghosh S, Guan C, Fries BL, Burke FW (2021): Acute on chronic theophylline toxicity in an elderly patient. *Cureus* **13**, e13484
- Lee SH, Sohn JT (2023): Mechanisms underlying lipid emulsion resuscitation for drug toxicity: a narrative review. *Korean J. Anesthesiol.* **76**, 171-182
<https://doi.org/10.4097/kja.23031>
- MacFarlane M (2009): Cell death pathways--potential therapeutic targets. *Xenobiotica* **39**, 616-624
<https://doi.org/10.1080/00498250903137990>
- Murad F, Shattuck Lecture (2006): Nitric oxide and cyclic GMP in cell signaling and drug development. *N. Engl. J. Med.* **355**, 2003-2011
<https://doi.org/10.1056/NEJMsa063904>
- Ohta K, Sawamoto S, Nakajima M, Kubota S, Tanaka Y, Miyasaka T, Nagai A, Hirai K, Mano K, Miyashita H (1996): The prolonged survival of human eosinophils with interleukin-5 and its inhibition by theophylline via apoptosis. *Clin. Exp. Allergy* **26**, 10-15
<https://doi.org/10.1111/j.1365-2222.1996.tb01137.x>
- Ohta K, Yamashita N (1999): Apoptosis of eosinophils and lymphocytes in allergic inflammation. *J. Allergy Clin. Immunol.* **104**, 14-21
[https://doi.org/10.1016/S0091-6749\(99\)70107-7](https://doi.org/10.1016/S0091-6749(99)70107-7)
- Ok SH, Yu J, Lee Y, Cho H, Shin IW, Sohn JT (2016): Lipid emulsion attenuates apoptosis induced by a toxic dose of bupivacaine in H9c2 rat cardiomyoblast cells. *Hum. Exp. Toxicol.* **35**, 929-937
<https://doi.org/10.1177/0960327115608930>
- Ok SH, Park M, Sohn JT (2020): Lipid emulsion treatment of nonlocal anesthetic drug toxicity. *Am. J. Ther.* **28**, e742-e746
<https://doi.org/10.1097/MJT.0000000000001300>
- Ok SH, Ahn SH, Lee SH, Kim HJ, Sim G, Park JK, Sohn JT (2022): Lipid emulsion attenuates propranolol-induced early apoptosis in rat cardiomyoblasts. *Hum. Exp. Toxicol.* **41**, 9603271221110852
<https://doi.org/10.1177/09603271221110852>
- Oxley-Oxland JV, Freercks R, Baker D, Van der Merwe E (2022): The characteristics and costs of severe theophylline toxicity in a tertiary critical care unit in Eastern Cape Province, South Africa. *S. Afr. Med. J.* **112**, 866-870
<https://doi.org/10.7196/SAMJ.2022.v112i11.16453>
- Park KE, Lee S, Bae SI, Hwang Y, Ok SH, Ahn SH, Sim G, Chung S, Sohn JT (2023): Theophylline-induced endothelium-dependent vasodilation is mediated by increased nitric oxide release and phosphodiesterase inhibition in rat aorta. *Gen. Physiol. Biophys.* **42**, 469-478
https://doi.org/10.4149/gpb_2023023
- Schulz M, Schmoldt A (2003): Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. *Pharmazie* **58**, 447-474
- Scurek M, Brat K (2024): A narrative review of theophylline: is there still a place for an old friend? *J. Thorac. Dis.* **16**, 3450-3460
<https://doi.org/10.21037/jtd-23-1781>
- Shehata SA, Abd El-Fadeal NM, Fattah IOA, Hagraas AM, Mostafa EMA, Abdel-Daim MM, Abdelshakour MA, Kolieb E, Abdelmaogood AKK, Rabee YM, et al. (2025): Synergistic cardiotoxic effects of captagon and azithromycin in rat via oxidative stress, apoptosis and upregulation of the PI3K/AKT/NF- κ B pathway. *Toxicol. Lett.* **408**, 77-94
<https://doi.org/10.1016/j.toxlet.2025.04.002>
- Sim G, Ok SH, Lee SH, Park KE, Park S, Sohn JT (2025): Lipid emulsion mitigates the cardiotoxic effects of labetalol in rat cardiomyoblasts. *Cells* **14**, 187
<https://doi.org/10.3390/cells14030187>
- Wang CH, Lin HC, Lin CH, Yu CT, Liu SL, Huang KH, Chung KE, Kuo HP (2003): Effect of theophylline and specific phosphodiesterase IV inhibition on proliferation and apoptosis of progenitor cells in bronchial asthma. *Br. J. Pharmacol.* **138**, 1147-1155
<https://doi.org/10.1038/sj.bjp.0705131>
- Wanten GJ, Calder PC (2007): Immune modulation by parenteral lipid emulsions. *Am. J. Clin. Nutr.* **85**, 1171-1184
<https://doi.org/10.1093/ajcn/85.5.1171>
- Yasui K, Agematsu K, Shinozaki K, Hokibara S, Nagumo H, Nakazawa T, Komiyama A (2000): Theophylline induces neutrophil apoptosis through adenosine A2A receptor antagonism. *J. Leukoc. Biol.* **67**, 529-535
<https://doi.org/10.1002/jlb.67.4.529>

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