<Supplementary material >

Thermogravimetric analysis

We used Thermo plus EVO II TG 8120 series thermal analyzer (RIGAKU, Tokyo, Japan) for the documentation of the curves for thermogravimetric analysis (TGA), differential thermal analysis (DTA), and derivative of TGA (DTG) of each solvent. All these three thermo-curves were recorded simultaneously within temperatures of 25–100 °C in an air atmosphere with the heating rates of 5 ℃/min. In all the thermo-curves, the sample mass initially weighed between 4–5 mg. The kinetic parameters were calculated using the data obtained from the recorded thermo-curves by employing non-mechanistic Kissinger evaluation method.

Analysis of gallstone components

The following assay kits were used for the analysis of gallstone components; Cholesterol Enzymatic Assay Kit (Cell Biolabs, San Diego, CA) for cholesterol, Triglyceride Quantification Kit (Cell Biolabs) for triglycerides, Phosphate Colorimetric Assay Kit (Biovision, Mountain View, CA) for phosphate, Bilirubin Assay Kit (Cell Biolabs) for bilirubin, Calcium Colorimetric Assay Kit (Biovision) for Calcium, and total bile acid kit (Cell Biolabs) for bile acid. Briefly, the gallstones were homogenized using the substances described in each assay kit and the supernatant was collected for assay. After incubation for a determined period at 25 °C in the assay buffer, the absorbance was measured using a microplate reader (model 680; Bio-Rad, Hercules, CA).

Cell viability assay

Cell viability of human gallbladder epithelial cells (hGBECs) was evaluated with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (water soluble tetrazolium salt; ST-1) assay using EZ-Cytox Cell Proliferation Assay kit (Itsbio, Seoul, Republic of Korea) according to the manufacturer's instructions. Additionally, we performed another series of cell viability assay for the following cells using Cyto X cell viability assay kit (LPS solution Co., Daejeon, Republic of Korea); Vero (African green monkey kidney, ATCC#81-CCL), L929 (mouse fibroblast, ATCC #2148-CRL), NIH 3T3 (mouse embryonic fibroblast, ATCC #1658-CRL), and CHI-K1 (Chinese hamster ovary, ATCC #61-CCL) cells.

Enzyme linked immunosorbent assay (ELISA)

After obtaining the sera from the hamsters at 24 h following solvent exposure, and the serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) concentrations were determined using ELISA kits (MyBioSource, San Diego, CA).

Preparation of human gallbladder epithelial cells

Human gallbladders were obtained after cholecystectomy performed at the Seoul St. Mary's hospital, the Catholic University of Korea. Human gallbladder epithelial cells (hGBECs) were isolated from the gallbladder by trypsin digestion method.[45] GBECs were maintained in DMEM/F12 medium (Thermo Scientific, Carlsbad, CA). The medium was supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT) and 1% penicillin-streptomycin antibiotics (Thermo scientific) at 37°C in a humidified atmosphere with 5% CO_2 in incubator.

Cell culture

Vero (African green monkey kidney, ATCC#81-CCL), L929 (mouse fibroblast, ATCC #2148-CRL), NIH 3T3 (mouse embryonic fibroblast, ATCC #1658-CRL), CHO-K1 (Chinese hamster ovary, ATCC #61-CCL) cells were used to assess cell viability. Cells were cultured in Dulbecco´s modified Eagle´s medium, supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin at 37℃ in a humidified incubator with 5% CO_2.

Western Blotting Analysis

The cells were washed two times with ice-cold phosphate buffer solution (PBS) and then lysed in EzRIPA Lysis kit (ATTO Corporation, Tokyo, Japan) which includes protease inhibitor and phosphatase inhibitor. After centrifugation at 12,000×g for 10 min, the supernatant was collected, and protein concentration was determined using Bradford reagent (Bio-Rad, Hercules, CA). Samples of equal amount of protein (30 μg) were loaded per well, separated on SDS-polyacrylamide gels, and then electrophoretically transferred onto nitrocellulose membranes. The membranes were then incubated with a blocking buffer (Biofact, Daejeon, Republic of Korea) at 23.5°C for 1 h. Subsequently, the membranes were incubated with primary antibodies (1:1,000 dilution) at 4°C overnight and then incubated with HRP-conjugated secondary antibodies (1:2,000 dilution) for 1 h at 23.5°C. The following antibodies were used: proliferation cell nuclear antigen (PCNA), myeloid cell leukemia 1 (Mcl-1), β-actin, HRP conjugated anti-rabbit IgG, and HRP conjugated anti-mouse IgG (All from Cell Signaling, Beverly, MA). Specific immune complexes were detected using Western Blotting Plus Chemiluminescence Reagent (Millipore, MA).

Immunohistochemical analysis

Paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated in a graded series of alcohol. The antigen was retrieved with 0.01 M citrate buffer (pH 6.0) by heating the sample in microwave oven for 10 min. The tissue sections were then placed in 3% hydrogen peroxide for 3 min to inactivate the endogenous peroxidase, and blocked for 10 min with normal horse serum in Vectastain ABC kit (Vector Laboratories, Burlingame, CA). The primary antibodies used for this study were cleaved caspase-3 and PCNA (All from MyBioSource). The pre-diluted primary antibodies were applied for overnight at 4℃. The slides were then treated with biotinylated secondary antibody for 20 min at 25°C, developed by immPACT^{TM}NovaRED^{TM} peroxidase substate kit (Vector Laboratories) for 10 min at 25°C.

***In vivo* acute toxicity test in mice**

Seven-week-old female Institute of Cancer Research (ICR) mice were purchased from Orient Bio, Inc. (Seongnam, Republic of Korea). The mice were allowed to acclimatize to their new environment for 7 days and were maintained in an environment with temperature 23 ± 3 °C, humidity 50 ± 10%, 12-hour light-dark cycle with 150–300 lux, and ventilation at 10–20 times/hour. For the acute toxicity study, the mice were divided into 3 groups: control (N = 7), MTBE (N = 14), and TAEE (N = 14). After starvation for 24 h, the control group was given 5% DMSO, while the treatment groups received MTBE and TAEE, respectively, at 2000 mg/kg prepared in a corn oil through oral gavage. The general behavior (skin, respiration, tremors, lethargy or sleep), body weight, and mortality were monitored for 14 days.

Measurement of Zebrafish locomotor activity according to each solvent

Zebrafish were maintained under standard conditions. To monitor the locomotor activity, larval zebrafish at 5 days post-fertilization were placed in individual wells of a 96-well plate with 100 μl embryonic medium. Then, 100 μl of MTBE and TAEE were added to a final concentration of 1 mM, respectively. After the treatment, changes were monitored at 28 °C for 60 minutes using DanioVision and EthoVision 10 XT locomotion tracking software (Noldus, Netherlands).