Supporting Information

Assessment of Dermal Safety of Scutellaria baicalensis Aqueous Extract Topical Application on Skin Hypersensitivity

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Experimental animals

Female BALB/c mice (16 to 18 g), male New Zealand white rabbits (2 to 3 kg), and male Hartley guinea pigs (250 to 300 g) were purchased from Orient Bio Korea and were acclimated for 1 week before beginning the experiments. They were housed at 20 ± 2 °C temperature, 55% ± 3% relative humidity, and 12-h light/dark cycle. Animals received tap water and a standard lab chow *ad libitum* throughout the study. The experimental protocols were approved (CNU-00156; 06. 2012) by the Institutional Animal Care and Use Committee of Chungnam National University (Daejeon, Korea).

Evaluation of cutaneous reaction

The IgE-dependent cutaneous reaction on female BALB/c mice ear was performed according to previous reports, with some modifications [18]. In brief, the mice were randomly divided into 6 groups (n = 7) as follows: normal (no treatment), control (distilled water [D.W.]), prednisolone, and WSBE group (0.5%, 1.0%, and 5.0% WSBE). Mice were treated topically with 20 µL of D.W. (control), 1% prednisolone (positive control; 99 %; Sigma-Aldrich), and 0.5%, 1.0%, or 5.0% WSBE in D.W. on each mouse ear lobe for 3 consecutive days. The 1% prednisolone was prepared by using 10% prednisolone in DMSO with dilution. At 3 h after the last treatment (except for the normal group), anti-DNP IgE was injected intravenously (250 ng/mouse, 250 µL; Sigma-Aldrich) for sensitization. After 24 h, the cutaneous reaction was induced by painting the ear lobe with 25 µL of 0.5% DNFB (Sigma-Aldrich) acetone-olive oil solution. Ear thickness was measured at 0 and 24 h after the DNFB challenge using a dial thickness gauge (Mitutoyo Corporation). Ears were then fixed immediately in a 10% buffered formalin phosphate solution, embedded in paraffin and cut into 5-µm sections. Four random sections for each skin sample were stained with hematoxylin and eosin (H&E) to
evaluate epidermal hyperplasia and infiltration of immune cells in the dermis. Image analysis for the quantification was performed using Image J (Rasband WS; ImageJ,) in 10 randomly-selected microscopic fields per specimen.

**Assessment of dermal irritation/corrosion**

The dermal irritation/corrosion effect of WSBE was assessed in accordance with the OECD guideline for the testing of chemicals (OECD TG 404, 2002). Three healthy male New Zealand white rabbits were used. Approximately 24 h before the test, the fur on the dorsolumbar region of each rabbit was clipped (approximate size, 12 cm²). The exposed area of skin was divided into 2 equal parts: one for vehicle control area and one for WSBE application. WSBE powder (0.5 g) was suspended in D.W. and applied to a 2.5-cm² gauze patch. Another patch was prepared with D.W to act as the vehicle control. After 4-h exposure of the patches to the rabbit skin, all patches were removed and any residue was wiped from the skin. The rabbits were observed at 1, 24, 48, and 72 h after patch removal to assess the degree of erythema, crust formation, and edema. If the reversibility still existed, observation was continued up to 14 days after removal of the patches. The skin irritation/corrosion test was scored according to the Draize scale. The primary irritation index (PII) was calculated using the mean score at 24, 48, and 72 h.

**Determination of skin sensitization**

Evaluation of the skin sensitization effect of WSBE was carried out using Buehler’s method, in accordance with the OECD guidelines for the testing of chemicals (OECD TG 406, 1992). Forty healthy male guinea pigs were used and randomly allocated into 3 groups: Group 1, WSBE treatment group (n = 20); Group 2, DNCB (97%, Sigma-Aldrich) in ethanol positive control group (n = 10); Group 3, D.W. treatment group (n = 10). Dose levels for the induction
and challenge phases were determined based on a preliminary study (data not shown).

The day before induction exposure, the left flank of each guinea pig was closely clipped (2.5 cm²) to expose the induction site. On day 0, test materials (0.4 g of WSBE powder in D.W., DNCB, and D.W. for each group) were fully loaded onto a gauze patch and tightly placed on the left flank for 6 h. After exposure, the patch was removed and the skin was gently washed with water. This process was repeated on days 7 and 14.

On day 28, the hair-free right flank of each animal was treated with an occlusive patch for challenge exposure for 6 h. Approximately 21 h after removing the patch, the challenge area was closely clipped for observation. The skin reactions were observed and scored at 24 and 48 h after the patch removal according to the Magnusson and Kligman grading scale.