Acute Dermal and Ocular Irritation Testing of Rice Bran Supercritical CO₂ Extract (RB-SCE) and 0.5% RB-SCE Essence Product

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Abstract

This study was conducted to investigate the acute dermal and ocular irritation potential of rice bran supercritical CO₂ extract (RB-SCE) and a 0.5% RB-SCE essence product in rabbits and guinea pigs. No abnormal clinical signs attributable to RB-SCE were detected. In the dermal irritation test, erythema, eschar, and edema formation was observed at 24 h of RB-SCE treatment, and the skin returned to normal after 72 h. The dermal primary irritation index (PII) of RB-SCE was 2.71; thus it was classified as a moderate irritant. In the ocular irritation test, there were no clinical signs related to the application of the RB-SCE; thus it was classified as a non-irritant according to the Draize scoring system. In the skin sensitivity test, there were no clinical signs related to the application of the RB-SCE; thus it was classified as a non-irritant according to the Draize scoring system. In a skin sensitivity test in rabbits, no abnormal clinical signs attributable to RB-SCE essence were observed and the PII was 0 because there was no evidence of erythema, eschar, or edema. In the guinea pig skin sensitivity test, no abnormal body weight changes or increased mortality was detected. Sensitization to the RB-SCE essence was 0%; thus it was classified as a very weak irritant.

Keywords: Rice bran, Supercritical CO₂ extract, Dermal irritation test, Ocular irritation test

Introduction

Rice (Oryza sativa) is one of the most important crops worldwide and rice bran is a major byproduct of the rice milling process. Rice bran is a brown layer that comprises nearly 8% of milled rice¹, production of which totals 50-60 million tons per year², with most used in animal feed and fertilizer. Depending on the milling procedure, bran contains 10-26% oil³, and 20-30% of produced rice bran is used for oil production⁴.

Rice bran is a foodstuff resource of free fatty acid, glycerides, sterols, proteins, and vitamins. In addition, it is a good resource of minerals such as silica, iron, calcium, and zinc⁵,⁶. Rice bran has received much attention because of its unique health benefits that may be attributed to the dietary fibre⁷ and the tocols⁸ present in it. Recently, it has also been reported that rice bran oil has various beneficial effects on ulcerative colitis⁹, cardiovascular disease¹⁰ and obesity¹¹.

Rice bran oil is typically extracted using an organic solvent such as hexane. However, hexane is highly volatile, flammable, and is considered toxic to animals and humans, even at low concentrations¹²,¹³. In addition, removal of residual hexane from rice bran oil is expensive and time-consuming¹⁴. Supercritical CO₂ extraction, which is conducted at low temperatures using supercritical CO₂ as the solvent, has been introduced as an alternative one-step oil and lipophilic compound extraction method. In addition, supercritical CO₂ is environmentally friendly, non-toxic, inflammable, inexpensive, and easily removed from the final product¹⁵.

Rice bran supercritical CO₂ extract (RB-SCE) has been shown to promote hair growth in vivo¹⁶ and to act as a 5-α-reductase inhibitor in cell lines in vitro¹⁷. Rice bran oil extracted via supercritical CO₂ is generally thought to be safe, although no toxicology studies for commercial applications have yet been performed. In addition, little information regarding the in vivo dermal and eye irritation activity of RB-SCE as a hair growth-promoting agent is available.

This study examined the potential dermal toxicological and ocular irritation effects of RB-SCE in New Zealand White (NZW) rabbits. We also performed skin sensitivity tests in NZW rabbits and guinea pigs of an
essence product containing 0.5% RB-SCE.

**Results**

**Ocular Irritation due to RB-SCE**

There were no significant changes in mortality, clinical signs, or body weight during the experimental period (data not shown). No changes attributable to RB-SCE were observed in the eye washing and no-eye-washing groups. Values for the individual ocular irritation index (IOII) and the mean index of ocular irritation (MIOI) indices were 0 in the G1 (eye washing group) and G2 (no eye washing group) for 7 days (Figure 1). Thus, the index of acute ocular irritation of RB-SCE at 7 days was 0, which indicates that RB-SCE is a non-irritant for eyes.

**Acute Dermal Irritation due to RB-SCE**

There were no significant changes in mortality, clinical signs, or body weight during the experimental period (data not shown). However, the body weight of all animals decreased slightly on day 1 (data not shown). On the intact skin of RB-SCE treatment sites of six animals, well-defined erythema (score = 2 in 1 animal) and moderate-to-severe erythema (score 3, in 5 animals) were observed after 24 h of treatment. Very slight (score 1), slight (score 2), and moderate (score 3) edema were observed in one, two, and two animals, respectively. In all abraded skin, moderate-to-severe erythema (score 3) and moderate edema (score 3) were observed after 24 h of treatment. After 72 h, no edema was observed; only one animal had very slight erythema (score 1) in the intact skin. However, eschar formation was noted in the abraded and intact skin of the treatment sites of all animals tested. The primary irritation index (PII) of RB-SCE was determined to be 2.71; it was thus classified as a ‘moderate irritant’ in this acute dermal irritation test. At the control site, no erythema, eschar or edema was observed in any animal after 24 and 72 h (Table 1, Figure 2).

**Skin Sensitivity Testing of RB-SCE Essence in Rabbits**

There were no mortality, clinical signs or significant body weight changes attributable to the application of RB-SCE essence in all animals. However, the body weight of all animals decreased slightly on day 1 (data not shown); however, no erythema, eschar or edema was observed at the RB-SCE essence treatment or control site (data not shown).

**Skin Sensitivity Testing of RB-SCE Essence in Guinea Pigs**

There were no abnormal clinical signs or body weight changes related to the administration of RB-SCE essence in guinea pigs (data not shown). No animals exhibited skin reactions in the vehicle control group or RB-SCE essence-treatment group; thus the sensitization rate was 0% for both groups. The skin sensitization potentials of these groups were classified as very weak (grade I). In the positive control group, 24 and