**Sparassis crispa** (Hanabiratake) Ameliorates Skin Conditions in Rats and Humans

Takashi Kimura, Mamiko Hashimoto, Munenori Yamada, and Yoshihiro Nishikawa

Research and Development Center, Unitika Ltd., 23 Uji-Kozakura, Uji, Kyoto 611-0021, Japan

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**Key words**: *Sparassis crispa* (SC); turnover of stratum corneum; soluble collagen; collagen synthetic activity-reduced model rat (RMR); transepidermal water loss (TEWL)

*Sparassis crispa* (SC) is an edible mushroom with various medicinal properties. In this study, we investigated to determine whether SC would affect skin conditions in rats and humans. Oral administration of SC increased both turnover of the stratum corneum and dermal soluble collagen content in collagen synthetic activity-reduced model rats. To investigate the effects of oral intake of SC in humans, we performed a randomized, double-blind, placebo-controlled study. We found that cheek transepidermal water loss was significantly lower in the experimental group than in the control group at 4 weeks of ingestion. This study suggests that SC is effective and safe for the improvement of skin conditions.

Rats**

**Table 1.** Composition of the Experimental Diet (%)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>6% Protein diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk casein*</td>
<td>7.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>63.0</td>
</tr>
<tr>
<td>Granulated sugar</td>
<td>10.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>6.0</td>
</tr>
<tr>
<td>Cellulose power</td>
<td>5.0</td>
</tr>
<tr>
<td>α-Starch</td>
<td>1.0</td>
</tr>
<tr>
<td>Vitamin mixb</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mixc</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*85% purity

**Abbreviations**: SC, *Sparassis crispa*; RMR, collagen synthetic activity-reduced model rat; TEWL, transepidermal water loss

Note

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*Sparassis crispa* (Wulf.) Fr. (SC), also known as cauliflower mushroom in English and hanabiratake in Japanese, is an edible mushroom with various medicinal properties that has recently been cultivated in Japan. It is a brown root fungus that grows primarily on the stumps of coniferous trees. It is widely distributed throughout the Northern Temperate Zone. More than 40% of dried SC consists of β-glucan, which is composed of a beta-(1→3)-β-glucan backbone with a single β-(1→6)-D-glucosyl side-branched unit occurring every three residues. SC has been reported to exhibit many biological activities, including tumor suppression,2–4) cancer prevention,5) enhancement of natural killer-cell activity,6) anti-angiogenic effects,7) anti-allergic effects,8) anti-diabetic effects,9) platelet anti-aggregation,9) HIV-1 reverse transcriptase inhibition,7) anti-hypertensive effects,8,9) and enhancement of hematopoietic responses.11) Moreover, oral administration of SC has been found to promote excisional wound healing in streptozotocin induced diabetic rodents.12,13) However, whether dietary supplementation with SC improves skin conditions in animals and humans remains unclear.

In this study, to evaluate the effects of SC on turnover of the stratum corneum and soluble collagen biosynthesis in dermis, we used collagen synthetic activity-reduced model rats (RMRs) fed a low-protein diet. Collagen synthetic activity in this animal model has been reported to be at the level observed in senescent rats.14) The composition of the experimental diet is shown in Table 1. All of the samples were prepared by Clea Japan (Tokyo). All animal procedures were performed following the ethical guidelines prescribed by the Animal Study Committee of Unitika Ltd. and the “Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain” (Notice no. 88, Ministry of the Environment, Government of Japan). Collagen synthesis was decreased by feeding of the animals with protein-deficient food, but this effect was more pronounced in younger animals.14)

In this study, 5-week-old male Wistar rats (CLEA Japan, Tokyo) were housed in standard stainless steel cages in an air-conditioned room (room temperature, 22 ± 1 °C; humidity, 55 ± 5% under a 12-h light/12-h dark cycle and were fed a 6% protein diet for 3 weeks ad libitum. After treatment, 8-week-old RMRs were obtained. Nine 8-week-old RMRs were divided into three groups. The control group was fed a 6% protein diet; the 70 mg group received oral administration of 70 mg of dried SC per kilogram of body weight per day in addition to the 6% protein diet; and the 210 mg group received oral administration of 210 mg of dried SC per kilogram of body weight per day in addition to the 6% protein diet. After 3 weeks of feeding, each RMR was administered 5 mL of 5% dansyl chloride (1-dimethylaminonaphthalene-5-sulphonyl chloride) ethanol solution on its back. The rate of desquamation was measured by dansyl chloride fluorescence,15) which was visually monitored with a UV lamp (UVGL-58, Funakoshi, Tokyo). The day when fluorescence completely disappeared was defined as the renewal time of the stratum corneum. The stratum

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1 To whom correspondence should be addressed. Tel: +81-774-25-2257; Fax: +81-774-25-2156; E-mail: takashi-kimura@unitika.co.jp

Abbreviations: SC, *Sparassis crispa*; RMR, collagen synthetic activity-reduced model rat; TEWL, transepidermal water loss
Organism against exogenous substances and preventing water loss.

The principle is simple and depends on fluorochrome labeling of the full thickness of the stratum corneum. The presence of fluorochrome is readily detected under ultraviolet illumination, and the duration it takes the fluorescence to disappear (renewal time) is assumed to be equal to the time it takes for the entire thickness of the stratum corneum to be exfoliated and replaced with new unstained cells from the dividing epidermis.

Neutral salt-soluble collagen (tropocollagen) in the dermal skin was extracted by homogenizing the skin and immediately stored at 4 °C until further analysis. The value marked by an asterisk was significantly different from that of control at p < 0.05.

Since it was first introduced by Jansen et al., the dansyl chloride labeling technique has become widely used as a method of measuring stratum corneum renewal time. The renewal time and Hyp content of the animals fed 210 mg of SC (210 mg/kg) demonstrated a significant reduction in renewal time as compared to the control group (Fig. 1A).

After an additional 2 weeks of feeding, all of the animals (13 weeks old) were sacrificed. The fur was shaved from the dorsum with electric clippers appropriate for use on small animals, and a 20-cm² piece of skin was obtained, weighed, frozen in liquid nitrogen, and immediately stored at −80 °C until further analysis. Neutral salt-soluble collagen (tropocollagen) in the dermal skin was extracted by homogenizing the skin in 0.14 M NaCl at 4 °C with a homogenizer (PT-10-35 GT, Kinematica, Luzern, Switzerland), shaking for 20 h at 4 °C, and then centrifuging the sample (10,000 rpm for 10 min), as previously described. The supernatant solutions were dialyzed against distilled water and then lyophilized and hydrolyzed with 6 N HCl at 110 °C for 24 h. t-Hydroxyproline (Hyp) is an amino acid that exists almost exclusively as a constituent of vertebrate collagen. The content of collagen may be deduced from the amount of Hyp. The Hyp content in the lyophilized residues was determined with an amino acid analyzer (Shimadzu, Kyoto, Japan). The amount of collagen in the skin was expressed as the Hyp content in 20 cm² of skin. The soluble collagen levels for the animals treated with 70 mg (70 mg/kg) and the 210 mg (210 mg/kg) of SC were 1.4 and 1.3 times higher respectively than that of the control group (Fig. 1B). This indicates that the levels of the newly synthesized collagen, which was hydrophilic and soluble, had increased.

Damage, such as that caused by natural aging and photoaging, causes cross-linking of collagen molecules, converting collagen into an insoluble form. This change in solubility contributes to the decrease in water content of the skin with age.

These results for the RMRs suggest that oral administration of SC exhibited positive effects on both the stratum corneum and dermal layer.

The renewal time and Hyp content of the animals fed the normal diet (20% protein) were approximately 7 d and 45 mg in 20 cm² of skin (data not shown). Next, to determine the effects of oral intake of SC in humans, we conducted a small-scale randomized, double-blind, placebo-controlled study that included 26 healthy volunteers in Japan, aged 20–60 years. The protocol of the study was approved by the Ethics Review Board of Unitika, and the study was performed in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants.

The subjects were divided into 2 groups: SC supplemented (n = 13) and placebo (n = 13). The material for oral supplementation contained 160 mg of SC dry powder and olive oil in the form of soft gel capsules. Identical placebo control capsules were prepared with lactose and olive oil in soft capsules. One capsule was orally administered to each subject twice daily. The testing period lasted for 4 weeks, and it began in February 2004. Measurements of transepidermal water loss (TEWL) were done at the beginning of the study and after 2 and 4 weeks. Measurements were done 30 min after the subjects were allowed to rest in a seated position in an environmental test room maintained at
20°C at 60% relative humidity. The TEWL of the left cheek was measured using Tewameter TM300 (Integral, Tokyo). Oral administration of SC (320 mg/day) for 28 consecutive days dramatically reduced transepidermal water loss, an indicator of the integrity of the skin barrier. In contrast, the placebo group showed no changes during the testing period (Fig. 2). Thus oral administration of SC had a positive effect on the skin barrier in humans.

It has been reported that oral administration of purified SC β-glucan has a suppressive effect on tumor growth and metastasis. In addition, recently we found that wound-healing activity occurred when purified SC β-glucan was administered topically to wounds of diabetic mice. We conclude that β-glucan of this mushroom probably contributes to these improvements in skin condition. But further studies are needed to identify the active component in SC definitely and to provide insight into the mechanism of this behavioral response.

In conclusion, we found positive effects induced by SC in the skin of rats with accelerated senescence and in healthy human volunteers. To the best of our knowledge, this is the first report suggesting that mushroom intake has a stimulatory effect on the stratum corneum. Moreover, many people in Japan have consumed SC, and to date no adverse events have been reported. Thus, SC appears to be effective and safe for the improvement of skin conditions.

References