Received: Jan 29, 2007
Accepted: May 19, 2007
Published online: June 1, 2007

Original Article

A double-blind clinical study of Rokkaku Reishi essence in women.

Yoshikazu YONEI [1)], Yoko TAKAHASHI [1)], Hozumi TAKAHASHI [1)], Miwako WATANABE [2)], Toshio MOCHIZUKI [3)], Hiroshi BANDO [4)]

1) Anti-Aing Medical Research Center, Doshisha University
2) Maressia Garden Clinic
3) Medical Urban Clinic
4) Department of Internal medicine, School of Medicine, Tokushima University

KEY WORDS: *Rokkaku Reishi* , anti-aging medicine, safety, oxidative-stress marker, skin

Abstract

A double-blind, randomized and placebo controlled study was carried out to examine the anti-aging efficacy and safety of Rokkaku Reishi in 22 overweight Japanese women (46.5±7.9 years old, BMI 26.8±2.7) consisting of 11 in the Analyte Group and 11 in the Placebo Group. Total cholesterol, LDL cholesterol and neutral fat values in the blood, which had been checked as background factors of the subjects, were mismatched, significantly higher respectively in the Analyte Group than in the Placebo Group. Evaluation of efficacy for lipid profiles was inevitable. In the Analyte Group the values of Hematocrit (–3.5%) and γ-GTP (–15.0%) significantly declined while HbA1c (2.3%) and K (5.4%) significantly increased after supplementation. The rate of increase of HbA1c in the Analyte Group was significantly higher than that in the Placebo Group. NK-cell activity showed no difference pre- and post-supplementation in either group. Oxidative-stress markers showed no significant difference pre- and post-supplementation for either group. A Common questionnaire on physical symptoms showed that the categories “Coughing and sputum” and “Grey hair” significantly declined after supplementation in the Analyte Group while no difference was found in the Placebo Group. For skin age scores measured using a Roboskinanalyzer, the scores for the categories “Noticeable pores”, “Large noticeable pores” and “Dusky noticeable pores” were significantly lower in the Analyte Group compared to the Placebo Group. A significant correlation was found between 8-OHdG in terms of its level, creatinine-corrected value and generation rate, and the number of “Noticeable pores”. No adverse reaction was found during the study. These results suggest that the four weeks supplementation of Rokkaku Reishi showed favorable effects on skin condition.

Introduction

The purpose of anti-aging medicine is to improve patient health and longevity. This type of health care aims not only to extend a patient’s life-span but also to promote health and happiness, maintain a high quality of life (QOL), and prevent the decline of the body due to aging [1-3)]. Anti-aging medicine can be categorized as a type of preventive medicine. Its therapeutic regimens include a complete aging checkup, life-long therapy such as the administration of supplements or hormone replacement therapy.

Recently, words such as comprehensive medical care, alternative healthcare, herbal remedy, acupuncture and moxibustion or anti-aging medical treatment have often been used with regard to describing anti-aging medicine. While various kinds of therapies are advocated owing to different perspectives based on traditional Western medicine, doctors have still not answered the question of to what extent they work and what symptoms or diseases they are effective against. This is due to the lack of use of common parameters in studies on anti-aging medicine in spite of many reports on its efficacy.

Several investigators have been using a common parameter to assess the effectiveness of comprehensive medical examinations and influence on the mind and body as well as safety issues and effects on obesity owing to supplements, health foods, medical devices, figure trimming equipment, and cosmetics, etc. [4-6)] Thanks to these studies, information on the levels or limitations of a product’s efficacy and on its spectrum of activity has been made available. In particular, anti-oxidant vitamins including vitamins A, C and E are becoming popular for use as supplements because they exhibit anti-aging activity through their ability to capture free-radicals which are produced as a result of oxidant stress. This study investigated the effect of the essence extracted from Rokkaku Reishi, which belongs to the Shelf Fungus family, on healthy middle-aged Japanese women. The essence of Rokkaku Reishi contains_-glucan, ganoderin acid, ganoderan and ganoderon. This study placed emphasis on the parameters that are considered to be important with regard to preventing aging from occurring. In addition, the effect of the essence on the QOL was also examined.

Professor Yoshikazu Yonei / Anti-Aging Medical Research Center / Doshisha University
1-3 Tatara-Miyakodani, Kyotanabe, Kyoto 610-0321, Japan
TEL: +81-75-391-5644 / e-mail: yyonei@mail.doshisha.ac.jp

Methods

·Analyte

The product (analyte) and the placebo selected for this study, which were offered by Pixen Corp. (Tokyo), were kept under appropriate storage conditions at the trial site, Medical Urban Clinic (Kobe). The analyte consisted of water, glycerin and Rokkaku Reishi. The daily dosage was set at 8 grams.

·Subjects

Twenty-two healthy but overweight Japanese women (46.5±7.9 years old, BMI 26.8±2.7) were selected in accordance with the following criteria.

1) Selection criteria
   1. Japanese woman aged between 30 and 59 years.
   2. Those with a BMI of 24 or greater.
   3. Those with the ability to fill out a personalized medical certificate.
   4. Those who were available to visit the appointed site on an appointed day.
2) Elimination criteria
   1. Those who were taking medications which could possibility influence the test results.
   2. Those who were consuming health foods daily which could possibility influence the test result.
   3. Those who were taking medications such as hormonal or osteoporotic agents.
   4. Those who were pregnant or may have been pregnant.
   5. Those who consumed excessive amounts of alcohol.
   6. Those who were at risk of being allergic to _-lipoic acid.
   7. Those who were participating in other clinical tests.
   8. Those who had chronic diseases such as diabetes, serious hepatic insufficiency, etc.
   9. Those who had serious disease of the liver, kidney, heart, blood or other serious complications.
   10. Those with severe anemia.
   11. Those for whom the doctor who was administering the test was found to be unsuitable.

Prior to the test, written consent was obtained from each subject at the trial site after a sufficient explanation was given regarding the following parameters before they were permitted to participate.

1. The objective and methods of the study.
2. An explanation about the analyte, its action and possible side-effects.
3. Each subject would be under the care of the doctor in charge of the study during their testing period.
4. Each subject would not suffer a loss due to their refusal to participate in the study.
5. Each subject could withdraw from the test at any time even after having given their consent to be examined.
6. Each subject would immediately receive information that may influence their will to continue participating in the study.
7. Parameters necessary for the protection of human rights and information discovery.
8. Protection of subject privacy in the case of publication of the study results.
9. Parameters that the subjects should observe including the general study schedule, times and dates of hospital visits, analyte and placebo doses, etc.
10. The provision of consultation services for subjects in medical institutions to which they would be referred to in the case of health hazards occurring that were related to the study as well as for those who wanted to obtain more information about the study or the rights of the participants.
11. Travel and cooperation expenses.

The subjects were divided into two groups of eleven women each at random, a Reishi (Analyte) Group and a Placebo Group. Verbal and written directions including the method of storage were provided when “Reishi” or the placebo were given to the subjects following their first observation.

The subjects were instructed to abstain from consuming food five hours before the test but to drink adequate fluids, to abstain from drinking the day before each observation, to avoid irregular living habits, to maintain the same quantity and quality of sleep, to diet and exercise as normal, to abstain from the use of health products except for “Reishi” which were designed to improve one’s health such as health foods, and to drink sufficient liquids after having blood withdrawn, etc.

·Protocol

This was a double-blind, randomized and placebo-controlled study. The subjects underwent basic physical measurements (weight : kg., height : cm., blood pressure : mmHg, pulse rate : /min., amount of body water : l., putative muscle bulk : kg., lean body weight : kg., amount of fat : kg., BMI, body fat percentage : kcal.) to obtain background factors twice during the observation period, at baseline, before the study, and at the end of the study, four weeks later.

All subjects were examined during each observation period with regard to hematological parameters including leukocyte count (/μl), erythrocyte count ($x10^4$ /μl), hemoglobin content (hemoglobin, g/dl), hematocrit level (%), and platelet count ($x10^4$/μl). The following blood biochemical parameters were also examined: total cholesterol (mg/dl), HDL-cholesterol (mg/dl), LDL-cholesterol (mg/dl), neutral fat (mg/dl), blood sugar (mg/dl), HbA1c(%), lipid peroxide (mmol/ml), CPK(IU/l), AST (GOT, IU/l), ALT(GPT, IU/l), γ-GTP (IU/l), BUN (mg/dl), ALP (IU/l), insulin (μU/ml), creatinine (mg/dl), NK-cell activity (%), CRP (mg/dl), total homocysteine (nmol/ml), uric acid (mg/dl), serum electrolyte (Na, K, Cl, mEq/l), insulin-like growth factor (IGF) -I (somatomedin C, ng/ml), and cortisol (μg/dl). In addition, urine parameters consisting of urine output (ml), creatinine (mg/dl), 8-hydroxy 2’–deoxyguanosine (8-OHdG, ng/ml) and isoprostanes (ng/ml) were investigated. The blood and urine tests were performed at the Anti-aging Test Department of Nikken SEIL Corporation and Mitsubishi Chemical BCL Inc. Using 8-OHdG [7-8] and isoprostanes [9-10] in the urine as indicators of oxidative stress, the rate of generation of 8-OHdG as well as isoprostanes and creatinine-corrected values (8-OHdG/CRE and isoprostanes/CRE) were calculated based on urine output and collection times, from the last urination the night before to the first urination in the morning, following measurements of 8-OHdG, isoprostanes and creatinine in the first urination volume early in the morning.

This study was performed with the approval of the ethics committee and in compliance with ethical principles based on the study protocol and the Helsinki Declaration as revised at the General Assembly in Edinburgh in 2000. This study also obeyed “An ethical principle about an epidemiologic study” by the

Ministry of Health, Labour and Welfare (http://www.niph.go.jp/wadai/ekigakurinri/ekigakurinrih170401/shishin-all.pdf). The human rights and the safety of the subjects as well as the reliability of the test results were safeguarded in accordance with the spirit of "Good Clinical Research Practice" (GCP) of the Ministry of Health and Welfare Law No.28, March 27th, 1997. The study period took place from October 21st to November 17th, 2005.

·Study and analysis of subjective symptoms

Physical and Mental symptoms were evaluated on a scale of 1 to 5 at baseline and at the end of the study using the Anti-aging QOL Common Questionnaire : AAQol.

·Analysis of skin age (digitized)

Photos were taken of each subject's face using a Roboskinanalyzer at baseline and at the end of the test at the test site. The photos were subsequently digitized to capture their skin age using an analysis system (Appendix 1) to help with the response evaluation.

·Safety evaluation

The degree of safety of the analyte depended on whether a hazardous incident occurred or not after supplementation started which would lead to discontinuation of the test or a subject being eliminated from the test. A hazardous incident was defined as a case where (1) physical signs or symptoms revealed "something new and uncommon" that would cause a clinical problem for the subject during the test period or "hatred" being recognized in a subject and (2) the doctor presiding over the test decided that a blood test had revealed abnormal changes. The doctor in charge of the test noted the type of incident on the case report form, the day of onset and disappearance, the degree of severity and content, the course of treatment taken, any follow-up studies and their outcome, causal relationships, and comments.

·Methods for statistics analysis

We used Excel Statistics (Social Information Service Corporation) statistical analysis software for data analysis. The t-test was performed to compare the back grounds of the Study Group and the Control Group. The paired t-test was used to compared the measured values between the level at baseline (A) and that after 4 weeks of supplementation (B) in each group. For inter-group comparison, the values of difference (B – A) was analyzed by Wilcoxon's signed rank test. The level of significance was set at 5 % or below. The relationship between each parameter was tested using Pearson's correlation coefficient.

Results

·Physical parameters (background factors)

Table 1 shows physical measurement values of the Analyte Group (11 subjects) and the Placebo Group (11 subjects) at baseline.

As seen in ***Table 1***, total cholesterol ($p<0.05$), LDL cholesterol ($p<0.05$), and neutral fat ($p<0.05$) levels as background factors in the blood were significantly higher in the Analyte Group compared with the Placebo Group. Evaluation for lipid profiles was excluded in this study.

Table 1 Physical parameters (background factors) of the subjects at baseline.

Parameter	Analyte Group	Placebo Group	p value
Number of subjects	11	11	
Age	47.0 ± 8.8	46.5 ± 8.1	
Weight (kg)	67.5 ± 8.5	64.9 ± 9.5	0.494
Height (cm)	157.2 ± 4.1	156.4 ± 5.3	0.698
BMI	27.3 ± 2.9	26.4 ± 2.5	0.450
Systolic BP (mmHg)	124.7 ± 22.7	118.6 ± 11.4	0.436
Diastolic BP (mmHg)	76.3 ± 15.6	74.6 ± 9.4	0.756
Pulse rate (/min.)	70.0 ± 8.3	71.9 ± 9.2	0.613
Neutral fat (mg/dl)	**151.6 ± 103.5**	**78.0 ± 26.1**	**0.043***
Total cholesterol (mg/dl)	**240.4 ± 37.7**	**209.6 ± 25.0**	**0.036***
LDL cholesterol (mg/dl)	**155.3 ± 28.0**	**126.8 ± 20.9**	**0.014***
HDL cholesterol (mg/dl)	59.7 ± 13.6	66.8 ± 15.5	0.268
Blood sugar (mg/dl)	86.6 ± 4.6	94.2 ± 17.4	0.185
Insulin (μU/mlÅj	7.86 ± 4.97	7.65 ± 6.89	0.936
HbA1c (%)	5.2 ± 0.4	5.3 ± 0.6	0.534

Average ± standard deviation, t-test. * p<0.05, ** p<0.01

2. Subjective symptoms

Table 2 shows scores for subjective symptoms indicated on the questionnaire pre- and post-supplementation with the analyte or the placebo.

Table 2-1 Scores for physical symptoms pre- and post-supplementation with the analyte

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
Tired eyes	3.18 ± 0.87	2.64 ± 1.12	0.111
Blurry eyes	2.36 ± 0.92	1.91 ± 1.04	0.053
Eye pain	1.91 ± 0.94	1.73 ± 1.19	0.506
Stiff shoulders	3.64 ± 1.12	3.00 ± 1.41	0.172
Muscular pain/stiffness	3.09 ± 0.94	2.91 ± 1.30	0.441
Palpitations	1.73 ± 0.79	1.73 ± 0.90	-
Dyspnea	2.00 ± 0.89	2.00 ± 1.00	-
Tendency to gain weight	4.64 ± 0.50	4.18 ± 1.17	0.176
Weight loss; thin	1.27 ± 0.47	1.09 ± 0.30	0.167
Lethargy	2.55 ± 1.29	2.27 ± 1.19	0.277
No feeling of good health	2.27 ± 0.79	2.27 ± 1.10	-
Thirst	2.27 ± 0.90	2.00 ± 0.89	0.391
Skin problems	2.64 ± 0.92	2.45 ± 0.93	0.617
Anorexia	1.18 ± 0.40	1.36 ± 0.67	0.441
Early satiety	1.73 ± 0.65	1.64 ± 0.67	0.756
Epigastralgia	1.45 ± 0.69	1.45 ± 0.69	-
Liable to catch colds	2.09 ± 1.14	1.91 ± 0.94	0.506
Coughing and sputum	**2.55 ± 1.04**	**1.73 ± 0.65**	**0.031** *
Diarrhea	1.55 ± 0.82	1.64 ± 0.92	0.676
Constipation	2.09 ± 1.14	1.64 ± 1.03	0.096
Gray hair	**3.91 ± 0.83**	**3.00 ± 1.34**	**0.016** *
Hair loss	3.18 ± 1.17	2.64 ± 0.92	0.082
Headache	2.55 ± 1.04	2.00 ± 1.18	0.140
Dizziness	2.09 ± 0.83	1.73 ± 0.79	0.221
Tinnitus	1.18 ± 0.40	1.27 ± 0.65	0.724
Lumbago	2.82 ± 1.25	2.55 ± 1.04	0.539
Arthralgia	2.18 ± 0.98	2.00 ± 1.18	0.617
Edematous	2.64 ± 1.03	2.45 ± 1.29	0.659
Easily breaking into a sweat	2.82 ± 1.40	2.91 ± 1.38	0.852
Frequent urination	2.18 ± 0.98	2.36 ± 0.81	0.341
Hot flash	2.18 ± 1.08	2.00 ± 0.89	0.506
Cold skin	2.82 ± 1.25	2.55 ± 1.21	0.518

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01

Table 2-2 Scores of physical symptoms pre- and post-supplementation with the placebo.

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
Tired eyes	3.18 ± 0.98	2.45 ± 1.44	0.070
Blurry eyes	2.55 ± 0.93	2.55 ± 0.93	-
Eye pain	1.55 ± 0.69	1.73 ± 1.19	0.341
Stiff shoulders	3.73 ± 1.35	3.36 ± 1.29	0.307
Muscular pain/stiffness	2.55 ± 1.04	2.64 ± 1.36	0.779
Palpitations	1.64 ± 0.81	1.64 ± 1.03	-
Dyspnea	1.64 ± 0.81	1.82 ± 0.98	0.167
Tendency to gain weight	4.27 ± 0.79	4.00 ± 1.26	0.277
Weight loss; thin	1.00 ± 0.00	1.00 ± 0.00	-
Lethargy	2.36 ± 0.67	1.91 ± 0.94	0.096
No feeling of good health	1.82 ± 0.60	1.55 ± 0.69	0.192
Thirst	1.82 ± 0.75	2.00 ± 0.77	0.506
Skin problems	2.91 ± 1.22	2.64 ± 1.29	0.432
Anorexia	1.91 ± 1.14	1.64 ± 1.03	0.557
Early satiety	1.91 ± 0.54	2.00 ± 0.89	0.676
Epigastralgia	1.82 ± 0.75	2.00 ± 0.89	0.341
Liable to catch colds	2.09 ± 0.83	2.00 ± 1.18	0.756
Coughing and sputum	1.82 ± 0.75	1.64 ± 1.03	0.506
Diarrhea	1.64 ± 0.67	2.00 ± 1.00	0.221
Constipation	1.91 ± 0.83	2.00 ± 1.00	0.779
Gray hair	2.82 ± 1.17	2.82 ± 1.17	-
Hair loss	2.36 ± 0.92	2.55 ± 1.13	0.506
Headache	2.64 ± 1.12	2.27 ± 1.35	0.307
Dizziness	1.73 ± 0.79	1.64 ± 0.92	0.676
Tinnitus	1.64 ± 1.21	1.55 ± 1.04	0.676
Lumbago	2.91 ± 1.58	2.55 ± 1.57	0.397
Arthralgia	2.18 ± 1.33	1.73 ± 0.79	0.138
Edematous	2.82 ± 1.25	2.64 ± 1.36	0.441
Easily breaking into a sweat	2.73 ± 1.56	2.27 ± 1.27	0.096
Frequent urination	2.00 ± 1.26	1.82 ± 1.08	0.341
Hot flash	1.82 ± 1.25	1.91 ± 1.14	0.588
Cold skin	2.45 ± 1.29	2.45 ± 1.21	-

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

Table 2-3 shows an inter-group comparison of differences pre- and post-supplementation with the analyte or the placebo for parameters which exhibited significant differences before and after.

Table 2-3 Comparison between the analyte and the placebo.

Parameter	Analyte Group	Placebo Group	p value
Number of subjects	11	11	
Coughing and sputum	–0.82 ± 1.08	–0.18 ± 0.87	0.144
Gray hair	**–0.91 ± 1.04**	**0.00 ± 0.63**	**0.023** *

Average ± standard deviation, Wilcoxon's signed rank test. * p<0.05, ** p<0.01.

Table 2-3 shows a decline in "Gray hair" (p<0.05) which was statistically and significantly greater for the Analyte Group than the Placebo Group. The decline in "coughing and sputum" after supplementation in the Analyte Group showed no difference from that of the Placebo Group .

Tables 2-4 and *2-5* show levels of "Mental symptoms" pre- and post-supplementation with the analyte or the placebo.

"Coughing and sputum" (p<0.05) and "Gray hair" (p<0.05) (*Table 2-1*) among "Physical symptoms" , and "Irritability"(p<0.05) among "Mental symptoms" were significantly improved after supplementation with the analyte . "Irritability" (p<0.01) and

Table 2-4 Scores of physical symptoms pre- and post-supplementation with the placebo.

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
Irritability	**2.73 ± 0.79**	**2.09 ± 1.14**	**0.026**
Easily angered	2.55 ± 0.93	2.18 ± 1.17	0.221
Loss of motivation	2.18 ± 0.60	2.09 ± 0.83	0.724
No feeling of happiness	1.91 ± 0.83	1.64 ± 0.67	0.082
Nothing to look forward in life	1.91 ± 0.94	1.82 ± 0.75	0.588
Daily life is not enjoyable	1.82 ± 0.60	1.64 ± 0.50	0.341
Loss of confidence	1.91 ± 0.83	1.82 ± 0.75	0.676
Reluctance to talk with others	1.55 ± 0.69	1.55 ± 0.69	-
Depressed	1.64 ± 0.67	1.55 ± 0.52	0.341
A sense of uselessness	1.73 ± 0.65	1.55 ± 0.52	0.167
Shallow sleep	2.00 ± 1.34	2.18 ± 1.33	0.588 *
Difficulty falling asleep	1.91 ± 1.30	2.00 ± 1.34	0.779
Pessimism	1.91 ± 0.83	1.73 ± 0.79	0.341
Lapse of memory	2.73 ± 0.79	2.64 ± 0.81	0.756
Inability to concentrate	2.18 ± 0.87	1.91 ± 0.70	0.277
Inability to solve problems	1.45 ± 0.69	1.64 ± 0.50	0.441
Inability to make judgments readily	1.55 ± 0.82	1.55 ± 0.52	-
Inability to sleep because of worries	1.64 ± 0.67	1.73 ± 0.65	0.676
A sense of tension	2.18 ± 0.87	2.00 ± 0.77	0.588
Feeling of anxiety for no special reason	1.73 ± 0.65	1.45 ± 0.52	0.082
A vague feeling of fear	1.36 ± 0.50	1.45 ± 0.52	0.588

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

Table 2-5 Scores of Mental symptoms pre- and post-supplementation with the placebo

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
Irritability	**2.45 ± 0.82**	**1.73 ± 0.65**	**0.004** **
Easily angered	**2.27 ± 0.65**	**1.73 ± 0.65**	**0.006** **
Loss of motivation	2.00 ± 0.77	1.73 ± 0.79	0.341
No feeling of happiness	1.91 ± 0.83	1.55 ± 0.52	0.104
Nothing to look forward in life	2.09 ± 1.04	1.55 ± 0.52	0.082
Daily life is not enjoyable	2.09 ± 1.04	1.64 ± 0.67	0.096
Loss of confidence	2.09 ± 1.22	1.55 ± 0.69	0.111
Reluctance to talk with others	1.64 ± 0.81	1.64 ± 0.67	-
Depressed	1.73 ± 0.79	1.64 ± 0.67	0.588
A sense of uselessness	1.82 ± 0.87	1.55 ± 0.69	0.277
Shallow sleep	2.45 ± 1.29	2.27 ± 1.19	0.617
Difficulty falling asleep	2.55 ± 1.13	2.27 ± 1.01	0.341
Pessimism	**2.18 ± 0.87**	**1.64 ± 0.67**	**0.025** *
Lapse of memory	2.64 ± 1.12	2.73 ± 1.01	0.676
Inability to concentrate	1.91 ± 0.70	1.91 ± 0.83	
Inability to solve problems	1.82 ± 0.60	1.64 ± 0.67	0.441
Inability to make judgments readily	1.82 ± 0.75	1.64 ± 0.67	0.341
Inability to sleep because of worries	1.82 ± 0.60	1.55 ± 0.52	0.082
A sense of tension	2.00 ± 0.89	1.91 ± 0.83	0.724
Feeling of anxiety for no special reason	1.36 ± 0.50	1.36 ± 0.50	-
A vague feeling of fear	1.27 ± 0.47	1.27 ± 0.47	-

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

"Pessimism" (p<0.05) among "Mental symptoms" were significantly improved after supplementation with the placebo while no change was found among "Physical symptoms". *Table 2-6* shows an inter-group comparison on differences pre- and post-supplementation with the analyte or the placebo concerning parameters which exhibited significant differences before and after.

Table 2-6 Comparison between the analyte and the placebo

Parameter	Analyte Group	Placebo Group	p value
Number of subjects	11	11	
Irritability	–0.64 ± 0.81	–0.73 ± 0.65	0.774
Easily angered	–0.36 ± 0.92	–0.55 ± 0.52	0.576
Loss of motivation	–0.18 ± 0.60	–0.55 ± 0.69	0.202

Average ± standard deviation, Wilcoxon's signed rank test. * p<0.05, ** p<0.01.

As seen in ***Table 2-6***, no significant difference was found in the inter-group comparison although improvements were noted for each parameter after supplementation with the analyte or the placebo.

3. Hematological parameters

Table 3-1 shows hematological parameters of the Analyte Group at baseline and after four weeks of supplementation while ***Table 3-2*** shows those of the Placebo Group.

Table 3-1 Hematological parameters for the Analyte Group

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
Leukocyte count (/μl)	6509.09 ± 1500.97	6381.82 ± 1160.02	0.772
Erythrocyte count (x10^4/μl)	483.36 ± 31.76	474.64 ± 30.58	0.156
Hemoglobin (g/dl)	13.71 ± 1.24	13.61 ± 1.10	0.586
Hematocrit (%)	**44.28 ± 3.02**	**42.74 ± 2.80**	**0.016** *
Platelet count (x10^4/μl)	25.77 ± 4.60	26.92 ± 5.75	0.122

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

Table 3-2 Hematological parameters for the Analyte Group

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
Leukocyte count (/μl)	6154.55 ± 1679.50	5972.73 ± 1627.32	0.515
Erythrocyte count (x10^4/μl)	427.64 ± 13.11	428.45 ± 18.35	0.876
Hemoglobin (g/dl)	12.95 ± 1.24	12.96 ± 1.16	0.953
Hematocrit (%)	41.14 ± 3.08	40.45 ± 2.77	0.136
Platelet count (x10^4/μl)	**26.59 ± 4.72**	**30.65 ± 7.69**	**0.013** *

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

As seen in ***Table 3-1***, the hematocrit level (p<0.05) significantly declined following supplementation with the analyte. ***Table 3-2*** shows that the platelet count (p<0.05) significantly increased following supplementation with the placebo. ***Table 3-3*** shows an inter-group comparison of parameters that exhibited significant differences pre- and post-supplementation with the analyte or the placebo.

Table 3-3 Comparison between the analyte and the placebo

Parameter	Analyte Group	Placebo Group	p value
Number of subjects	11	11	
Hematocrit (%)	–1.55 ± 1.77	–0.69 ± 1.41	0.225
Platelet count (x10^4/μl)	1.15 ± 2.25	4.06 ± 4.47	0.067

Average ± standard deviation, Wilcoxon's signed rank test. * p<0.05, ** p<0.01.

As seen in ***Table 3-3***, there was no significant difference between the Analyte Group and the Placebo Group for each parameter.

4. Blood biochemical parameters

Table 4-1 shows the results of blood tests at baseline and after four weeks of supplementation in the Analyte Group. ***Table 4-2*** shows that for the Placebo Group.

Table 4-1 Blood biochemical parameters of the Analyte Group

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
AST(GOT)(IU/l)	23.55 ± 11.23	22.45 ± 11.60	0.391
ALT(GPT)(IU/l)	24.82 ± 20.80	22.55 ± 20.18	0.182
γ-GTP (IU/l)	**24.18 ± 11.20**	**20.55 ± 8.19**	**0.049** *
BUN (mg/dl)	12.82 ± 3.37	12.45 ± 2.98	0.700
ALP (IU/l)	212.18 ± 43.60	218.09 ± 46.30	0.105
HbA1c (%)	**5.18 ± 0.35**	**5.30 ± 0.38**	**0.005** **
Insulin (μU/ml)	7.86 ± 4.97	15.35 ± 20.79	0.165
Creatinine (mg/dl)	0.64 ± 0.10	0.65 ± 0.11	0.461
Uric acid (mg/dl)	4.87 ± 0.79	4.94 ± 0.88	0.708
Total cholesterol (mg/dl)	240.36 ± 37.65	223.91 ± 33.74	0.051
HDL cholesterol (mg/dl)	59.73 ± 13.58	57.91 ± 15.08	0.444
LDL cholesterol (mg/dl)	155.27 ± 28.03	144.64 ± 28.74	0.110
Neutral fat (mg/dl)	151.55 ± 103.51	142.27 ± 138.75	0.681
Blood sugar (mg/dL)	86.55 ± 4.63	90.64 ± 8.59	0.066
Lipid peroxide (nmol/ml)	0.97 ± 0.44	1.18 ± 0.69	0.151
CPK (IU/l)	98.00 ± 44.20	98.64 ± 50.91	0.946
NK-cell activity (%)	41.31 ± 22.36	41.32 ± 17.32	0.998
CRP (mg/dl)	0.15 ± 0.16	0.15 ± 0.10	-
Total homocysteine (nmol/ml)	9.49 ± 2.33	9.05 ± 1.94	0.118
Na (mEq/l)	140.00 ± 1.48	140.18 ± 1.33	0.749
K (mEq/l)	**3.91 ± 0.24**	**4.12 ± 0.21**	**0.003** **
Cl (mEq/l)	102.00 ± 2.00	102.91 ± 1.45	0.176
IGF-I (ng/ml)	202.55 ± 78.29	213.00 ± 90.83	0.329
DHEA-S (μg/dl)	161.09 ± 71.14	152.91 ± 59.91	0.260
Cortisol (μg/dl)	9.29 ± 4.67	8.72 ± 3.47	0.714

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

Table 4-2 Blood biochemical parameters of the Placebo Group

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
AST(GOT)(IU/l)	22.91 ± 11.63	20.18 ± 4.21	0.413
ALT(GPT)(IU/l)	23.55 ± 10.95	20.36 ± 7.49	0.230
γ-GTP (IU/l)	22.00 ± 16.63	20.73 ± 17.29	0.100
BUN (mg/dl)	11.82 ± 2.89	11.73 ± 2.90	0.908
ALP (IU/l)	208.09 ± 56.45	222.36 ± 55.91	0.056
HbA1c (%)	5.32 ± 0.62	5.34 ± 0.61	0.441
Insulin (μU/ml)	7.65 ± 6.89	10.23 ± 8.09	0.213
Creatinine (mg/dl)	0.63 ± 0.09	0.63 ± 0.09	0.590
Uric acid (mg/dl)	4.53 ± 0.91	4.47 ± 0.69	0.784
Total cholesterol (mg/dl)	209.64 ± 25.01	208.91 ± 22.51	0.898
HDL cholesterol (mg/dl)	66.82 ± 15.54	64.36 ± 12.82	0.188
LDL cholesterol (mg/dl)	126.82 ± 20.91	122.27 ± 21.43	0.376
Neutral fat (mg/dl)	78.00 ± 26.06	98.45 ± 49.44	0.151
Blood sugar (mg/dL)	94.18 ± 17.35	96.55 ± 16.02	0.608
Lipid peroxide (nmol/ml)	**0.82 ± 0.08**	**1.07 ± 0.23**	**0.004** **
CPK (IU/l)	479.09 ± 1131.33	122.45 ± 48.36	0.322
NK-cell activity (%)	47.28 ± 17.21	48.48 ± 14.74	0.690
CRP (mg/dl)	0.09 ± 0.05	0.10 ± 0.06	0.676
Total homocysteine (nmol/ml)	14.07 ± 14.06	14.05 ± 14.72	0.954
Na (mEq/l)	139.82 ± 1.72	139.45 ± 2.02	0.441
K (mEq/l)	3.96 ± 0.36	4.15 ± 0.36	0.074
Cl (mEq/l)	102.00 ± 1.55	102.00 ± 2.05	-
IGF-I (ng/ml)	173.64 ± 44.04	169.73 ± 52.94	0.709
DHEA-S (μg/dl)	140.64 ± 56.74	141.55 ± 49.75	0.928
Cortisol (μg/dl)	8.50 ± 4.11	9.83 ± 3.31	0.092

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

Table 4-1 shows that HbA1c ($p<0.05$) and K ($p<0.01$) significantly rose after supplementation with the analyte. *Table 4-2* shows that lipid peroxide ($p<0.01$) significantly rose after supplementation with the placebo. However, no influence was found on NK cell activity in the immune system. *Table 4-3* shows an inter-group comparison of parameters with significant differences pre- and post-supplementation with the analyte and the placebo.

Table 4-3 Comparison between the Analyte Group and the Placebo Group

Parameter	Analyte Group	Placebo Group	p value
Number of subjects	11	11	
γ-GTP(IU/l)	–3.64 ± 5.37	–1.27 ± 2.33	0.196
HbA1c (%)	**0.12 ± 0.11**	**0.02 ± 0.08**	**0.020** *
Lipid peroxide (nmol/ml)	0.21 ± 0.45	0.25 ± 0.23	0.767
K (mEq/l)	0.21 ± 0.18	0.19 ± 0.32	0.870

Average ± standard deviation, Wilcoxon's signed rank test. * p<0.05, ** p<0.01.

Table 4-3 shows that the rise in HbA1c was significantly greater after supplementation in the Analyte Group compared with the Placebo Group.

5. Change in oxidative stress levels

Table 5 shows levels of oxidative-stress markers and related parameters at baseline and after four weeks of supplementation with the analyte or the placebo. *Tables 5-1* and *5-2* show no significant change in any parameter after supplementation with the analyte or the placebo.

Table 5-1 Oxidative stress marker levels pre- and post-supplementation with the analyte.

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
8-OHdG level(ng/ml)	4.44 ± 3.24	6.68 ± 4.68	0.208
Isoprostanes(ng/ml)	2.08 ± 1.54	3.36 ± 2.85	0.072
Creatinine(mg/dl)	58.0 ± 37.4	88.1 ± 55.3	0.053
8-OHdG/CRE(ng/mgCRE)	7.30 ± 2.62	8.08 ± 3.99	0.384
Isopostanes/CRE(ng/mgCRE)	3.56 ± 1.75	3.44 ± 1.56	0.457
8-OHdG generation rate(ng/kg/hr)	4.07 ± 3.06	5.46 ± 3.71	0.338
Isoprostanes generation rate(ng/kg/hr)	1.76 ± 1.06	2.79 ± 2.45	0.102
Urine output(ml)	364.6 ± 116.9	370.9 ± 97.0	0.763
Urine collection time(hr)	6.05 ± 1.52	6.41 ± 1.11	0.349

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

Table 5-2 Oxidative stress markers pre- and post-supplementation with the placebo.

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
8-OHdG level(ng/ml)	8.09 ± 3.56	9.30 ± 4.93	0.540
Isoprostanes(ng/ml)	3.33 ± 1.94	4.26 ± 3.90	0.297
Creatinine(mg/dl)	109.3 ± 53.0	108.8 ± 61.7	0.976
8-OHdG/CRE(ng/mgCRE)	7.97 ± 2.98	8.80 ± 2.83	0.314
Isoprostanes/CRE(ng/mgCRE)	3.08 ± 1.13	3.48 ± 2.07	0.371
8-OHd generation rate (ng/kg/hr)	6.00 ± 2.29	6.86 ± 5.04	0.634
Isoprostanes generation rate(ng/kg/hr)	2.33 ± 0.88	3.09 ± 3.47	0.469
Urine output(ml)	297.3 ± 115.7	329.6 ±162.3	0.372
Urine collection time(hr)	5.91 ± 1.87	6.59 ± 1.24	0.207

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

6. Analysis of skin age

Tables 6-1 and *6-2* show skin age scores pre- and post-supplementation with the analyte or the placebo.

Table 6-1 Skin age scores pre- and post-supplementation with the analyte.

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
Moisture level (0-100)	61.27 ± 4.67	56.27 ± 12.81	0.233
Oil level (0-100)	9.45 ± 9.18	8.45 ± 7.89	0.782
Skin color tone (0-360)	57.22 ± 1.00	56.97 ± 0.72	0.197
Skin clearness (0-100)	**48.79 ± 5.46**	**46.96 ± 5.07**	**0.002** **
Skin lightning (0-100)	61.25 ± 3.23	61.81 ± 3.45	0.053
Pigmentation(small)number	33.00 ± 11.54	28.73 ± 10.65	0.071
Pigmentation(big)number	**83.27 ± 33.30**	**72.36 ± 29.61**	**0.010** **
Pigmentation(small)dimension (mm^2)	24.18 ± 8.78	21.00 ± 8.09	0.092
Pigmentation(big)dimension (mm^2)	307.64 ± 160.85	259.91 ± 152.46	0.066
Noticeable pores (number)	**1941.18 ± 614.89**	**1255.64 ± 428.07**	**0.000** **
Large noticeable pores(number)	**138.27 ± 78.90**	**55.73 ± 33.25**	**0.000** **
Dusky noticeable pores(number)	**1008.27 ± 422.94**	**557.09 ± 233.77**	**0.000** **
Delicateness (0-100)	16.45 ± 7.88	16.09 ± 9.26	0.908
Wrinkles under the right eye(number)	5.82 ± 2.68	5.00 ± 2.68	0.353
Wrinkles under the left eye(number)	5.82 ± 3.25	5.36 ± 2.77	0.583

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

Table 6-2 Skin age scores pre- and post-supplementation with the placebo

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
Moisture level (0-100)	68.27 ± 8.53	65.91 ± 11.02	0.297
Oil level (0-100)	10.27 ± 9.42	13.64 ± 12.09	0.357
Skin color tone (0-360)	55.72 ± 4.16	55.36 ± 5.66	0.563
Skin clearness (0-100)	**47.76 ± 6.08**	**46.69 ± 6.18**	**0.029** *
Skin lightning (0-100)	61.44 ± 2.62	61.34 ± 2.16	0.876
Pigmentation(small)number	24.91 ± 7.63	24.36 ± 10.02	0.832
Pigmentation(big)number	61.64 ± 28.20	56.45 ± 25.45	0.052
Pigmentation(small)dimension (mm^2)	17.55 ± 5.70	17.73 ± 7.42	0.926
Pigmentation(big)dimension (mm^2)	**237.55 ± 123.70**	**193.00 ± 102.07**	**0.020** *
Noticeable pores (number)	**1433.36 ± 873.03**	**1030.27 ± 887.68**	**0.000** **
Large noticeable pores(number)	**98.91 ± 91.72**	**61.18 ± 82.81**	**0.001** **
Dusky noticeable pores(number)	**730.09 ± 573.58**	**511.00 ± 567.00**	**0.000** **
Delicateness (0-100)	18.45 ± 7.63	15.36 ± 8.09	0.435
Wrinkles under the right eye(number)	4.09 ± 1.70	4.45 ± 1.37	0.307
Wrinkles under the left eye(number)	6.27 ± 2.61	5.36 ± 1.57	0.194

Average ± standard deviation, paired t-test. * p<0.05, ** p<0.01.

The "Pigmentation number" (big) ($p<0.01$), the number of "Noticeable pores" ($p<0.01$), the number of "Large noticeable pores" ($p<0.01$) and the number of "Dusky noticeable pores" ($p<0.01$) significantly declined after supplementation with the analyte, while "Skin clearness" ($p<0.01$) significantly decreased (*Table 6-1*). In addition, the number for "Pigmentation (big)" ($p<0.05$), the number of "Noticeable pores" ($p<0.01$), the number of "Large noticeable pores" ($p<0.01$) and the number of "Dusky noticeable pores" ($p<0.01$) significantly declined after supplementation with the placebo, while "Skin clearness" ($p<0.05$) (*Table 6-2*) significantly decreased. *Table 6-3* shows an

inter-group comparison of differences pre- and post-supplementation with the analyte or the placebo, concerning parameters which had significant differences before and after.

Table 6-3 Comparison between the analyte and the placebo.

Parameter	Before supplementation	After 4 weeks of supplementation	p value
Number of subjects	11	11	
Skin clearness (0-100)	–1.83 ± 1.48	–1.07 ± 1.40	0.233
Skin lightning (0-100)	0.56 ± 0.85	–0.10 ± 2.07	0.337
Pigmentation(small)number	–10.91 ± 11.40	–5.18 ± 7.78	0.184
Pigmentation(big)dimension (mm2)	–47.73 ± 76.59	–44.55 ± 53.44	0.911
Noticeable pores (number)	**–685.55 ± 354.29**	**–403.09 ± 208.55**	**0.034** *
Large noticeable pores(number)	**–82.55 ± 53.68**	**–37.73 ± 26.30**	**0.022** *
Dusky noticeable pores(number)	**–451.18 ± 269.30**	**–219.09 ± 116.77**	**0.016** *

Average ± standard deviation, Wilcoxon's signed rank test. * p<0.05, ** p<0.01.

As seen in ***Table 6-3***, declines in the number of "Noticeable pores" ($p<0.05$), the number of "Large noticeable pores" ($p<0.05$) and the number of "Dusky noticeable pores" ($p<0.05$) were statistically and significantly greater in the Analyte Group. ***Table 7*** shows the correlation between skin age scores and oxidative-stress markers.

Table 7 shows that a significant correlation existed between "Noticeable pores" among the skin age scores and 8-OHdG level ($p<0.05$) in the urine, its creatinine-corrected value ($p<0.05$) or its generation rate ($p<0.05$).

7. Safety

No report of hazardous incidents was made by the subjects in the Analyte Group or the Placebo Group. Furthermore the clinical study showed no abnormal changes in the subjects.

Table 7 Correlation between oxidative-stress marker and skin age scores

		Moisture level (0-100)	Skin lightning level (0-100)	Pigmentation(small) dimension(mm^2)	Noticeable pores(number)	Dusky noticeable pores(number)
8-OHdG level (ng/ml)	**Pearson's correlation coefficient**	–0.194	0.523	0.600	0.661*	0.652*
	Significance probability (two-sided)	0.567	0.099	0.051	0.027	0.0309
Isoprostanes (ng/ml)	**Pearson's correlation coefficient**	–0.013	0.558	0.171	0.426	0.3832
	Significance probability (two-sided)	0.970	0.074	0.616	0.191	0.2447
Creatinine(CRE) (mg/dl)	**Pearson's correlation coefficient**	–0.304	0.604*	0.486	0.471	0.4574
	Significance probability (two-sided)	0.364	0.049	0.130	0.1437	0.1588
8-OHdG/CRE (ng/mgCRE)	**Pearson's correlation coefficient**	0.124	0.269	0.471	0.608*	0.5988
	Significance probability (two-sided)	0.7175	0.423	0.143	0.047	0.0523
Isoprostanes/CRE (ng/mgCRE)	**Pearson's correlation coefficient**	0.673*	–0.1773	–0.342	–0.451	–0.2766
	Significance probability (two-sided)	0.023	0.603	0.303	0.164	0.4119
8-OHdG generation rate (ng/kg/hr)	**Pearson's correlation coefficient**	–0.404	0.572	0.621*	0.634*	0.5691
	Significance probability (two-sided)	0.218	0.066	0.042	0.036	0.0687
Isoprostanes generation rate (ng/kg/hr)	**Pearson's correlation coefficient**	–0.105	0.586	0.141	0.420	0.3646
	Significance probability (two-sided)	0.759	0.0582	0.679	0.198	0.2727

*; p <0.05.

Table 1. Biomarker levels before and after 4 weeks supplementation with Lingzhi (*Ganoderma lucidum*) or placebo (*n* 18; fasting samples)* (Mean values and standard errors of the mean)

	Placebo				Lingzhi			
	Day 1		Day 29		Day 1		Day 29	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma FRAP (μmol/l)	1125	42	1148	37	1151	45	1091	38
Urine FRAP (mmol/μmol creatinine)	434	52	411	43	329	37	415	74
Plasma ascorbic acid (μmol/l)	57·2	2·8	59·8	3·7	59·9	5·2	56·9	4·5
Plasma α-tocopherol (μmol/l)	24·8	1·07	24·1	1·1	22·6	1·0	22·6	1·4
Lipid standardised α-tocopherol (μmol/mmol TC + TG)	3·89	0·19	3·72	0·19	3·99	0·13	4·19	0·18
Plasma TC (mmol/l)	5·22	0·24	5·35	0·27	4·75	0·26	4·31	0·12
Plasma TG (mmol/l)	0·91	0·08	1·05	0·10	0·94	0·10	0·85	0·09
Plasma HDL-cholesterol (mmol/l)	1·48	0·08	1·45	0·10	1·48	0·10	1·42	0·08
Plasma LDL-cholesterol (mmol/l)	3·12	0·29	3·61	0·32	2·76	0·23	2·59	0·20
hsCRP (mg/l)	0·91	0·23	0·67	0·12	0·83	0·21	0·87	0·18
Plasma uric acid (μmol/l)	341	24	350	17	320	19	335	21
Erythrocyte SOD (U/g Hb)	1183	23	1187	28	1265	23	1274	24
Erythrocyte GPx (U/g Hb)	50·9	3·2	47·9	2·5	45·7	2·8	45·4	2·5
MDA (μmol/l)	0·93	0·05	0·97	0·05	1·31	0·07	1·38	0·08
Percentage DNA in comet tail (baseline)	5·5	0·7	5·9	0·8	5·6	0·9	5·2	0·4
Percentage DNA in comet tail (after 15 μM-H_2O_2 oxidant challenge)	11·0	0·8	10·2	0·7	11·2	0·9	10·3	0·9
CD4:CD8 ratio	1·6	0·2	1·5	0·1	1·5	0·1	1·6	0·2
Leucocytes ($\times 10^9$/l)	6·06	0·3	6·59	0·3	5·98	0·3	6·27	0·3
ALT (U/l)	18·8	3·05	19·2	3·05	19·3	2·56	18·4	1·98
AST (U/l)	26·2	1·72	28·3	1·96	21·9	1·98	23·8	2·78
Creatinine (μmol/l)	84·8	4·4	80·1	7·2	79·4	7·0	80·6	5·9

FRAP, ferric-reducing/antioxidant power; TC, total cholesterol; TG, triacylglycerol; hsCRP, high-sensitivity C-reactive protein; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde; ALT, alanine transaminase; AST, aspartate aminotransferase.
* For details of procedures, see p. 264.

Discussion

·The effect of "Rokkaku Reishi"

"*Reishi*", a mushroom whose Japanese name is "*Mannenntake*" (Scientific name : *Ganoderma lucidum*), belongs to the Shelf Fungus family. It has come to be called "*Rokkaku Reishi*" because its sprouts look similar to "*Rokkaku*" or "deer horns". In China, people have call it "*Lingzhi*", and consider it to be an "elixir of life" from ancient times. Ganoderma ludicum has been reported to possess characteristics such as anti-aging [12] and anti-oxidant activities, the ability to reduce blood sugar and cholesterol [18-20], the ability to act as an immunoenhancer, and to protect DNA. However, only a few clinical studies involving this mushroom have been conducted on humans.

The Wachtel-Galor [13] group found that the anti-oxidant potential in the blood plasma and the urine became elevated within three hours after having administered 1.1~3.3 grams of *Lingzhi* once to ten healthy volunteers. After this, they [25] could not obtain results that showed a significant difference between the *Ganoderma ludicum* group and the Placebo Group in terms of oxidation state, DNA damage, immunity or inflammatory condition following a double-blind, cross-over and a placebo-controlled intervened study, which they performed for four weeks using 18 healthy volunteers. (Ref. the table below)

·Activity of Rokkaku Reishi in this study

The declining rate of "White hair" and decreasing rate of "Noticeable pores" were significantly greater in the Rokkaku Reishi Group than in the Placebo Group in this double-blind, randomized, placebo-controlled study, which was performed for four weeks using healthy middle-aged Japanese women. In addition, there was a significant correlation between the how noticeable their pores were and 8-OHdG, an oxidative-stress marker. These results show that *Rokkaku Reishi* may delay skin aging by controlling of oxidative stress. Evidence of this using the objective data on white hair could not be found.

It has been suggested that Rokkaku Reishi acts as an anti-diabetic agent because pretreatment prevents with it alloxan-induced pancreatic islet damage [16]. Furthermore, it causes declines in blood sugar in a dose-dependent manner[17] following a single dose administration of 25, 50, and 100 mg/kg *Rokkaku Reishi* as seen through an interperitoneal using healthy mice. However, the significant decline in blood sugar that was seen between three to six hours disappeared after twelve hours in that study. This study found no change in insulin or glucose levels in the blood, while the increase in HbA1c in the *Rokkaku Reishi* group was significant. It is not appropriate to relate the rise of HbA1c seen in this study to the effect of *Rokkaku Reishi* because HbA1c reflects the blood sugar levels of the last one to two months.

There is no outstanding issue regarding the safety of *Rokkaku Reshi* because no subject reported a hazardous incident in this study and no hazardous incident was noted in the Wachtel-Galor's study [25].

In conclusion, these results suggest that the four weeks supplementation of *Rokkaku Reishi* showed favorable effects on skin condition. They also suggest that there are no safety issues surrounding short-term administration.

References

1) Yonei Y, Mizuno Y. The human dock of tomorrow -Annual health checkup for anti-aging-. Ningen Dock 19:5-8, 2005.
2) Yonei Y, Iwaita Y, Muramatsu K, Mizuno Y. The anti-aging secrets of Japanese executives. Anti-Aging Medical Research 2:61-69, 2005.
3) Yonei Y, Mizuno Y, Kido M, Kaku L, Yasuda H. Research on toxic metal levels in scalp hair of the Japanese. Anti-Aging Medical Research 2:11-20, 2005.
4) Bando H, Yoshioka T, Yonei Y, Nakamura T. Investigation of quality of life in athletes from an anti-aging perspective. Primary Care Jpn 4:47-51, 2006.
5) Yonei Y, Mizuno Y, Togari H, Sato Y. Muscular resistance training using applied pressure and its effects on the promotion of growth hormone secretion. Anti-Aging Medical Research 1:13-27, 2004.
6) Yonei Y, Mizuno Y, Katagiri E. Effects of cosmetics therapy using isoflavone and pine bark extract on the skin and QOL: A double-blind placebo-controlled trial.Anti-Aging Medical Research 1:48-58, 2004.
7) Floyd RA, Watson JJ, Wong PK, Altmiller DH, Rickard RC. Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. Free Radic Res Commun 1:163-172, 1986.
8) Toyokuni S, Tanaka T, Hattori Y, Nishiyama Y, Yoshida A, Uchida K, Hiai H, Ochi H, Osawa T. Quantitative immunohistochemical determination of 8-hydroxy-2'-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrilotriacetate-induced renal carcinogenesis model. Lab Invest 76:365-374, 1997.
9) Morrow JD, Awad JA, Kato T, Takahashi K, Badr KF, Roberts LJ 2nd, Burk RF. Formation of novel non-cyclooxygenase-derived prostanoids (F2-isoprostanes) in carbon tetrachloride hepatotoxicity. An animal model of lipid peroxidation. J Clin Invest 90:2502-2507, 1992.
10) Milne GL, Morrow JD. Isoprostanes and related compounds: update 2006. Antioxid Redox Signal 8:1379-1384, 2006.
11) Kawada A, Kameyama H, Asai M, Shiraishi H, Aragane Y, Tezuka T, Iwakiri K. A new approach to the evaluation of whitening effect of a cosmetic using computer analysis of video-captured image. J Dermatol Sci 29:10-18, 2002.
12) Chen K, Li C. Recent advances in studies on traditional Chinese anti-aging material medica. Journal of Traditional Chinese Medicine 13:223-226, 1993.
13) Wachtel-Galor S, Szeto Y-T, Tomlinson B, Benzie IF. Ganoderma lucidum ('Lingzhi'); acute and short-term biomarker response to supplementation. Journal of Food Science and Nutrition 55:75-83, 2004.
14) Sun J, He H, Xie BJ. Novel antioxidant peptides from fermented mushroom Ganoderma lucidum. Journal of Agricultural Food Chemistry 52:6646-6652, 2004.
15) Wong KL, Chao HH, Chan P, Chang LP, Liu CF. Antioxidant activity of Ganoderma lucidum in acute ethanol-induced heart toxicity. Phytotherapy Research 18:1024-1026, 2004.
16) Zhang HN, He JH, Yuan L, Lin ZB. In vitro and in vivo protective effect of Ganoderma lucidum polysaccharides on alloxan-induced pancreatic islets damage. Life Sciences 73:2307-2319, 2003.
17) Zhang H-N, Lin Z-B. Hypoglycemic effect of Ganoderma lucidum polysaccharides. Acta Pharmacologica Sinica 25:191-195, 2004.
18) Berger A, Rein D, Kratky E, Monnard I, Hajjaj H, Meirim I, Piguet-Welsch C, Hauser J, Mace K, Niederberger P. Cholesterol-lowering properties of Ganoderma lucidum in vitro, ex vivo, and in hamster and minipigs. Lipids in Health and Disease 3:2, 2004.
19) Hajjaj H, Mace C, Roberts M, Niederberger P, Fay LB. Effect of 26-oxygenosterols from Ganoderma lucidum and their activity as cholesterol synthesis inhibitors. Applied and Environmental Microbiology 71:3653-3658, 2005.
20) Chen WQ, Luo SH, Li HZ, Yang H. Effects of ganoderma lucidum polysaccharides on serum lipids ad lipoperoxidation in experimental hyperlipidemic rats (Chinese). Zhongguo Zhong Yao Za Zhi 30:1358-1360, 2005.
21) Kohguchi M, Kunikata T, Watanabe H, Kudo N, Shibuya T, Ishihara T, Iwaki K, Ikeda M, Fukuda S, Kurimoto M. Immuno-potentiating effects of the antler-shaped fruiting body of Ganoderma lucidum (Rokkaku-Reishi). Bioscience Biotechnology and Biochemistry 68:881-887, 2004.
22) Lin Z-B. Cellular and molecular mechanisms of immuno-modulation by Ganoderma lucidum. Journal of Pharmacological Science 99:144-153, 2005.
23) Pero RW, Amiri A, Sheng Y, Welther M, Rich M. Formulation and in vitro/in vivo evaluation of combining DNA repair and immune enhancing nutritional supplements. Phytomedicine 12:255-263, 2005.
24) Wachtel-Galor S, Choi S-W, Benzie IFF. Effect of Ganoderma lucidum on human DNA is dose dependent and mediated by hydrogen peroxide. Redox Report 10:145-149, 2005.
25) Wachtel-Galor S, Tomlinson B, Benzie IFF. British Journal of Nutrition 91:263-269, 2004.

Appendix 1: Skin pictures analysis system (Parameters and outline of the test used for skin analysis).

Explanation about the measurement of parameters used for analyzing the skin pictures

Definition of "moisture"

Moisture level (0-100)

The moisture level of the skin was determined through the electrical capacitance of a sensor which was applied directly to it. Normal saline was considered to be in a "saturated condition (100) and the absence of water was said to be the "zero condition (0)".

Oil level (0-100)

We could determine the area covered by the oil through the refractive index of a pressure sensor which was applied to the surface. This permitted the dimensions of the oil to be measured. The condition whereby the surface was entirely covered with oil was considered to be the "saturated condition (100), and the absence of oil was the "zero condition (0)".

Definition of "skin color"

First, we created forty measuring ranges in total on both cheeks directly under the eyes. Next, we measured three color parameters, brightness, tone and clearness, for each measurement range. Then we ranked the results of each of the parameters in order of magnitude. Last, the average of the middle-ranked twenty dimensions was decided on as the final result.

Skin lightning level (0-100)

This term was used to denote the whiteness of the skin. Improvement of skin darkness resulted in an increased skin lightning level.

< Suntan causes a decrease in the skin lightning level.>

Skin color tone (0-100)

This was expressed using an angle. A numerical value close to 0°(0°or close to 360°) meant that the skin had a very reddish color tone while a relatively high numerical value (about 45°) meant that the skin had a very strongly yellowish color tone.

< Sunburned skin shows a low value while it shows high value after setting down.>

Skin clearness (0-100)

This is a numerical value which expressed the clearness of the skin color or blood circulation and dullness of the skin. A low value (20 and below) represented brownish dull skin caused by blood circulation in the skin having become hidden by a thick stratum corneum. A high value (75 or over) indicated dullness-free beautiful skin.

Definitions of “pore” and “pigmentation”

Pores and pigmentations were expressed by tone and shape properties on black-and-white pictures made by using a BLUE signal because pores and pigmentations are clearly observed especially in the “BLUE” signal element among the three color elements that exist in color pictures.

Noticeable pores

Roundish and continuous areas which measured 0.1~0.6mm2 for each were detected as “Noticeable pores” among objects whose rims could be detected as “a little dark part” or “dark parts” compared with the peripheral parts in a black-and-white picture.

Large noticeable pores

Roundish and continuous areas which measured 0.3~0.6mm2 for each were detected as “Large noticeable pores” among objects whose rims could be detected as “ little dark parts” or “dark parts” compared with peripheral parts in a black-and-white picture.

Dusky noticeable pores

Roundish and continuous areas which measured 0.1~0.6mm2 for each were detected as “Dusky noticeable pores” among objects whose rims could be detected as “dark parts” compared with peripheral parts in a black-and-white picture.

Pigmentation (small)

Continuous areas which measured 0.6~1.2mm2 were detected as “pigmentation (small)” among objects whose rims could be detected as “little dark parts” or “dark parts” compared with peripheral parts in a black-and-white picture.

Pigmentation (big)

Continuous areas which measured over 1.2mm2 were detected as “pigmentation (big)” among objects whose rims could be detected as “little dark parts” or “dark parts” compared with peripheral parts in a black-and-white picture.

Definition of “wrinkle”

Fifty to sixty lines were drawn within a parallelogram which lay under each eye between 9 and 15mm from the eye. Dark areas with dense and sharp inclinations against the lines were detected as “wrinkles under eyes”. The densest lines with wrinkles among multiple detected lines were counted as representative lines except for detected lines with obstructions such as eyelashes.

Definition of “delicateness”

It was decided that the dark area would be called a skin groove and the light area would be called a skin ridge in a black-and-white picture. The finalized results after the light parts and dark parts were processed, respectively, were emphasized. For this, the nearer the delicate model of the equilateral triangle, the higher the score on the test which had a maximum score of 100.

Test outline for skin analysis

Skin analyses of the subjects were performed based on the table below.

Measuring instrument	**Roboskinanalizer manufactured by Infoward corp.**	
Component of the examination	Moisture level	Moisture level (0-100)
	Oil level	Oil level (0-100)
	Skin lightning level	(0-100)
	Pigmentation (small)	Number
	Pigmentation (big)	
	Pigmentation (small)	Dimension (mm^2)
	Pigmentation (big)	
	Pores	Number
	delicateness	(0-100)
	Wrinkles	Number of wrinkles under the eyes
Outcome measure	See attached sheet	
Inspection measure	Each subject’s face was photographed using the Roboskinanalyzer prior to being analyzed. Inspections were performed twice, before and after the application.	
Data	Full-face picture	
	Magnified picture of skin delicateness of a designated area	