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Isaria sinclairii Extract Reduces Body Weight and Ameliorates Metabolic Abnormalities

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Obesity is a major risk factor for cardiovascular disease. In our case study using animal models for disease states such as obesity or hypertension, we found that, *Isaria sinclairii* remarkably reduced body weight and ameliorated metabolic abnormalities in Zucker and SHR rats.

Genetically obese (fa/fa) Zucker rats were one animal model chosen for this study. Four groups of rats received a standard diet and were treated orally with the following test samples daily for 8 weeks: saline (negative control), ethanol extracts of *I. sinclairii*, hot water extract of *I. sinclairii*, or Xenical® (30 mg/kg, positive control). Mild reductions (6.3%) in body weight gain were observed in the groups treated with the hot water extract of *I. sinclairii* compared to the control after 8 weeks. Interestingly, organ weight was greatly reduced by this Dongchunghacho (*I. sinclairii*), in parallel with the mild reductions in body weight gain and reductions in abdominal fat (adipose tissue). Also observed was a 4.1% decrease in the ratio of heart weight/body weight compared to the control group. As a hypertensive animal model, SHR (spontaneously hypertensive rat) and WKY (Wistar Kyoto) rats were also administered these extracts for one month. Treatment with the hot water extract of *I. sinclairii* caused greater reductions in body weight gain for the SHR group (10.9%) compared to the WKY group's (5.2%).

Based on these results, *I. sinclairii* extracts contain selective action for anti-obesity activity, naturally occurring candidate for regulation of body weight increase, as demonstrated in the present study.

Key words: Antiobesity, *Isaria sinclairii, Zucker rat, SHR*

Introduction

Obesity is the most prevalent and serious nutritional disease in developed countries, and is rapidly replacing under nutrition as the most common form of malnutrition throughout the world (Kushner, 2002). Growing evidence suggests that obesity initiates a cascade of disorders, including hypertension (Shaper, 1996), diabetes (Poonawala *et al.*, 2000), arteriosclerosis (Hall *et al.*, 2002), cancer (Jee *et al.*, 2006), and aging (Slawik and Vidal-Puig, 2006). To control obesity, anti-obesity food ingredients may avert the disease, possibly leading to the prevention of lifestyle-related maladies, if the ingredients are effective in reducing body fat accumulation (Saito *et al.*, 2005). The primary goal of obesity treatment is weight loss. However, obesity is associated with metabolic disorders, which also need to be taken into consideration (Vikramadithyan *et al.*, 2003).

Medicinal mushrooms, which are recognized for their many health benefits, have received increasing attention from the pharmaceutical industry (Wang *et al.*, 2003). Among the Dongchunghacho, Cicada Dongchunghacho (*Isaria sinclairii*) has recently been introduced in a powdered form, as a potential crude drug in Korea. This Dongchunghacho contains the fruiting bodies of *I. sinclairii*, and sometimes its parasitic host larva (silkworm). Furthermore, FTY720, the semi-synthetic derivative of

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Myriocin (ISP-1) from *I. sinclairii* cell supernatant, acts as a safe and potent immunomodulator by decreasing numbers of peripheral lymphocytes, especially CD4 positive cells and IL-2R positive cells (Ueda *et al.*, 2005). Moreover, this Dongchunghacho possesses selective action for antihypertensive activity in spontaneously hypertensive rats (SHR) (Ahn *et al.*, 2007).

During our previous anti-hypertensive animal study in SHR/WKY rats, we found that this particular Dongchunghacho possesses a potent anti-obesity effect. Therefore, we tried to ascertain this effect in obese Zucker rata as well. At the time of this study, there were no data on the anti-obesity efficacy or safety of *I. sinclairii* within rats, especially among normal rats (SD and WKY rats) and spontaneous animal models for human diseases (SHR and, Zucker rats). Hence, the present investigation was undertaken to assess the biological activity, particularly the anti-obesity activity (prevention of body weight gain) of *Isaria sinclairii* in four strains of rats, including SHR, WKY, Zucker-fa/fa and Sprague-Dawley (SD) rats.

Materials and methods

Materials

I. sinclairii was collected at Mountain Halla, located in South Korea. This fungus endophytically parasitizes on dead or living *Cicadae* spp. Our particular strain was isolated from conidiospores and cultured in a potato dextrose agar (PDA) medium, and then sprayed (inoculated) on silkworms for infection. Through evasion of each of the defensive mechanisms employed by the host insect, either during penetration of the cuticle or upon reaching the hemocoel, *I. sinclairii* proliferated inside the insect body to form fruiting bodies, which were cultivated at the Department of Agricultural Biology, National Institute of Agricultural Science and Technology, Korea. Xenical (Orlistat) was purchased from Roche Korea (Seoul) and is manufactured by F. Hoffmann-La Roche.

Preparation of *I. sinclairii* extract

The dried *I. sinclairii* (50 g) was homogenized in a blender, and then soaked with deionized water or methanol for the extraction. The samples were filtered with Whatman paper, and concentrated by evaporating, and then freeze-dried. The dried powders (water/methanol extracts) were dissolved in saline as test solutions.

Animals

The Zucker-fa/fa rat (5-week- old, males) strain, which is genetically obese due to an impaired regulation of leptin expression in the stomach (Pico *et al.*, 2002), was originally procured from Tokyo University School of Medicine and is now used extensively in obesity studies; the rats used in this study were supplied by Japan SLC Co. (Shizuoka, Japan). All procedures were done in accordance with the NIH Guidelines for Care and Use of Laboratory Animals. Before any testing began, the rats were acclimated for one weeks to normal physical conditions ($23 \pm 2°C$, $55 \pm 10\%$, humidity, and regular day/night cycle): they were fed a standard diet (Samyangsa, Korea) and provided water *ad libitum*. At 6 weeks of age, Zucker-fa/fa rats with body weights ranging from 190 to 200 g were randomly divided into control and treatment groups ($N = 6$ for each group). The control rats received one oral treatment of saline per day. The treated groups received 100 mg/kg of *I. sinclairii* ethanol extracts (ISMC), 100 mg/kg of *I. sinclairii* hot water extract (ISHW), or Xenical® (30 mg/kg) daily, for 2 months.

Nine-week-old male spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats, weighing 200 ± 5 g, were supplied by Japan SLC Co. (Shizuoka, Japan) and divided into the following four groups of 10 rats: SHR control group, *I. sinclairii*-treated SHR groups, WKY control group, and *I. sinclairii*-treated WKY groups. Again, the treatment groups received ISMC (100 mg/kg), ISHW (100 mg/kg) or Xenical® (30 mg/kg) for 2 months. Sprague-Dawley (SD) male rats were also treated similarly and received the same doses as the SHR/WKY rats for 2 weeks.

Body weight

The body weight of each rat was measured at the initiation of treatment, and once a week thereafter, and finally again on the day of scheduled autopsy. Changes in body weight were recorded weekly for every group, and the means were calculated.

Food consumption

Food consumption was measured on a per-cage basis at the start of treatment and at weekly intervals thereafter. The amount of food was measured before it was supplied to each cage, and the difference at the end of the week was calculated as the daily food consumption (g/rat/day).

Organ weights

The absolute and relative (organ-to-body weight ratio) weights of the following organs were measured in all rats upon autopsy: adrenal glands, liver, spleen, kidneys, heart, thymus, lung, salivary glands, thyroid gland, testes, epididymis, seminal vesicles, and prostate.

Statistical analysis

Mean and standard error values of all the parameters stud-

Table 1. Comparison of mean % change in body weight of Zucker obese rats orally treated with *I. sinclairii* extract for 8 weeks

Change in weight (%)	Con (Saline)	MC100	HW100	Xe30	Non-treat
M±SE	72.05±5.92	69.56±5.62*	67.52±5.44*	70.47±5.67*	84.63±6.23
(%)	100	96.54	93.71	97.81	117.53

MC100 : methanol extract of *I. sinclairii*, 100 mg/kg; HW100 : hot water extract of *I. sinclairii*, 100 mg/kg; Xe30 : Xenical 30 mg/kg MC100, HW100 and Xe30, *$p<0.05$, compared to control (saline group).

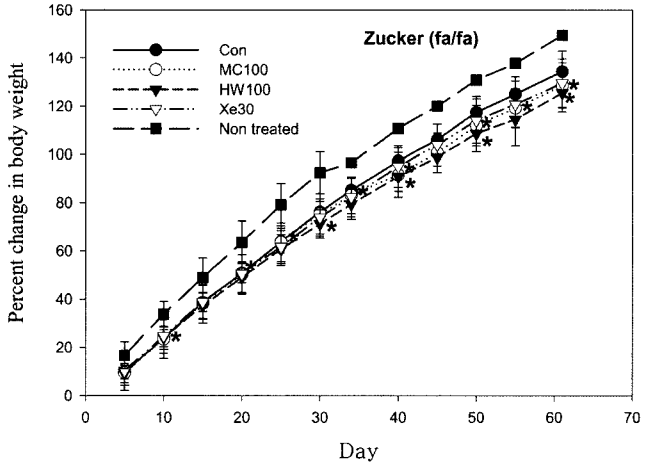

Fig. 1. Body weight increase rate (%) of *I. sinclairii* extracts during 2 months in obese Zucker rats.
Con : saline; MC100 : methanol extract of *I. sinclairii*, 100 mg/kg; HW100 : hot water extract of *I. sinclairii*, 100 mg/kg; Xe30 : Xenical 30 mg/kg. Each value represents mean±S.D. MC100, HW100 and Xe30, *$p<0.05$, compared to control (saline group).

ied were determined for each group. Student's *t*-tests were conducted to establish significant differences between the final biochemical levels in the control versus treated groups. A level of $p<0.05$ was considered statistically significant.

Results

Clinical signs and food consumption

No deaths or adverse clinical signs were observed as a result of the treatments with *I. sinclairii* in the Zucker, SHR, WKY, and SD rats. Food consumption did not differ between the groups during the course of the study (data not shown.).

Body Weight changes in obese Zucker rats over a 2-month period

During the testing period, the body weights of the male Zucker-fa/fa rats (6 to 15 weeks of age) in the 2 *IS* treatment groups increased less than those of controls (Fig. 1). This reduction in body weight gain was gain was observed in the *I. sinclairii*-treated groups after 2 weeks. Interestingly, when comparing the mean percentage in body weight, the group treated with the hot water extract (100 mg/kg) of IS (HW100) exhibited a significantly shallower curve than the groups treated the methanol extract (100 mg/kg) of IS (MC100), the Xenical (30 mg/kg) (Xe30), or the control. That is, body weight gains were significantly reduced in the following order: ISHW100 >IS MC100>Xenical (30 mg/kg) versus control, when comparing the mean percentage in body weight: HW100, 6.3% decrease, MC100, 3.4% decrease, Xe30, 2.2% decrease (Fig. 1, Table 1).

Body weight changes compared to various rat strains and extract method

There are many difference of body weight increase rate between disease oriented rat and normal rats, or extraction method by *I. sincrairii* extract treatment. In case of SHR (hypertension disease model rat), two-weeks of oral treatment, the body weights gains were also significantly reduced (8.7%) by the administration of ISHW100 (Table 2). Furthermore, anti-obesity efficacy of hot water extract of *I. sinclairii* (ISHW) was superior to methanol extract (ISMC), which showed only slight body weight gain reductions (−4.8%) when treated with 100 mg/kg.

However, the case of normal rats, the body weights gains of Wistar Kyoto rats (WKY) were reduced less sig-

Table 2. Body weight changes according to treated rat strains

Rat species (Treat Period)	Body weight loss (%)			
	HW100	MC30	MC100	Xe30
SHR (2 week)	8.7	5.0	4.8	–
SHR (1 month)	–	10.9	–	–
WKY (1 month)	–	5.2	–	–
SD (2 week)	−1.9*	−1.3	−1.1	2.9
Zucker (8 week)	6.3		3.5	2.2

MC30 : *I.S.* methanol extract 30 mg/kg; MC100 : *I.S.* methanol extract 100 mg/kg; HW100 : hot water extract of *I. sinclairii*, 100 mg/kg; Xe30 : Xenical 30 mg/kg. - : not tested.
*$p<0.05$, compared to control (saline group).

nificantly than the weight gains of SHR by one-month of treatment of ISMC100 groups (Table 2). That is, the WKY group exhibited greater reductions in body weight (BW) gain (5.2%, BW loss),) than the SHR group receiving ISMC30 (10.9%, BW loss). Also, in normal Sprague-Dawley (SD) rats over a 2-week period, we attained a significantly smaller percentage of body weight gain reduction compared to the other rat models of disease [Zucker (not shown), SHR, and WKY] (Fig. 2). Therefore, body weight increased (1.1 to 1.3% BW increase) at *I. sinclarii* treated SD rat group at 30 mg/kg, or at 100 mg/kg, in the SD rat group. On the other hand, the hot water extract (ISHW100) caused slightly greater (−1.9%) reductions rather than the methanol extract of IS. The rats treated

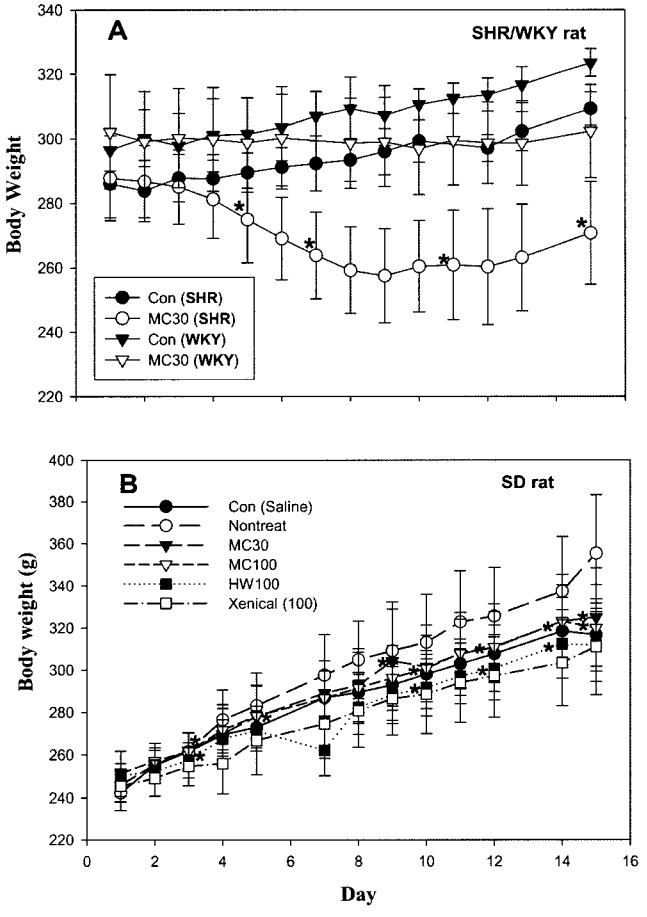

Fig. 2. A) Body weight change of oral treatment of *I. sinclairii* extracts during a month in spotaneously hypertensive rats and Wistar Kyoto rats. MC30 : methanol extract of *I. sinclairii*, 30 mg/kg; Each value represents mean ± S.D., $*p<0.05$, compared to control.

B) Body weight change of *I. sinclairii* extracts during 2 weeks in SD male rats. MC30 or MC100 : methanol extract of *I. sinclairii*, 30 or 100 mg/kg; HW100 : Hot water extract of *I. sinclairii*, 100 mg/kg; Xe100 : Xenical 100 mg/kg. Each value represents mean ± S.D., $*p<0.05$, compared to control.

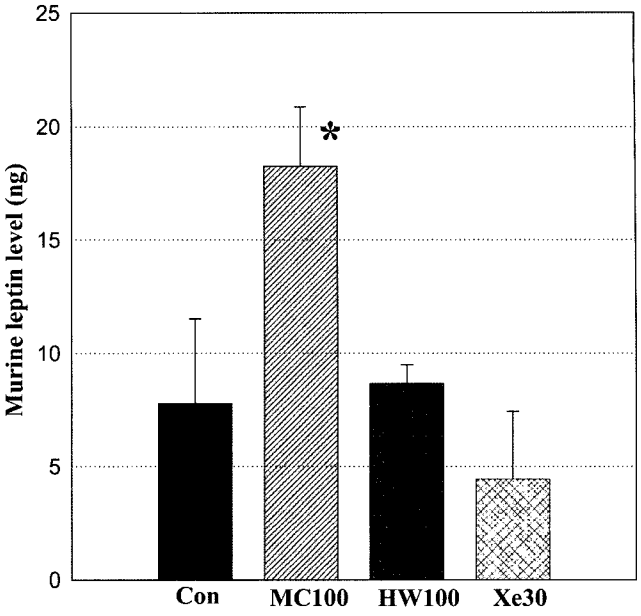

Fig. 3. Murine leptin level (ng) of *I. sinclairii* extracts during 2 months in obese Zucker rats after 2 month administration period. Con : saline; MC100 : methanol extract of *I. sinclairii*, 100 mg/kg; HW100 : hot water extract of *I. sinclairii*, 100 mg/kg; Xe30 : Xenical 30 mg/kg. Each value represents mean ± S.D. $*p<0.05$, compared to control (saline group).

with Xenical experienced a marked 2.9% body weight loss (Table 2). The body weight changes data, according to each treated rat strains are summarized in Table 4.

Quantification of Serum Leptin Levels

Serum levels of leptin in obese (fa/fa) Zucker rats showed 7.79 ± 3.74 (ng/ml) before treatment of ISMC. After treatment of ISMC100, however, serum leptin levels were markedly increased by 134% in ISMC100 treated groups. On the other hand, the other groups treated with Xe30 (Xenical 30 mg/kg) failed to elevated serum leptin levels (Fig 3). A good inverse relationship was shown between increased levels of serum leptin and decreased Body weight (g).

Pathology and organ weight

There were no significant treatment-related pathologies; and there were few minor changes that were dose independent. In accordance with their defatted bodies, the absolute organ weights of the Zucker rats at the end of the administration period were slightly lower compared to the controls. Within the cardiac system, the absolute and relative cardiac weights decreased slightly for all groups [methanol extract of I.S. (−1.9%), hot water extract (−4.1%), Xenical (−1.8%)] (Table 3). However, there were no significant differences compared to the control group in the absolute organ weight changes by the treatment with *I.*

Table 3. Absolute heart weight of Zucker obese rats orally treated with *I. sinclairii* for 8 weeks

Mean±SD	Control (Saline)	MC100	HW100	Xe30
n	6	6	6	6
Heart weight (g)	1.17±0.03	1.11±0.07	1.12±0.07	1.11±0.05
Heart (g)/kg BW	2.26±0.06	2.17±0.14	2.14±0.13	2.16±0.09

MC100 : methanol extract of *I. sinclairii*, 100 mg/kg; HW100 : hot water extract of *I. sinclairii*, 100 mg/kg; Xe30 : Xenical 30 mg/kg. Each value represents mean±S.D.

sinclairii extract (100 mg/kg) for 2 months.

Discussion

Obesity is a progressive disease of unwanted fat accumulation that has multiple organ-specific pathological consequences, and is associated with causing various diseases including diabetes, stroke, cancer, tiredness and heart disease (Lean *et al.*, 2000). To control obesity, energy intake must be limited and lipid metabolism, including hormone metabolism and excretion, should be regulated. Generally, strong intraspecies correlations have been observed within rats across multiple exposure routes (Delisraty, 2000). With Regard to *I. sinclairii* extracts, there have been rat and dog acute toxicity reports (Ahn *et al.*, 2003 and 2004), and a genotoxicity report (Ahn *et al.*, 2004). Yet, no data were found for its anti-obesity efficacy or safety within rats, especially between normal rats (SD and WKY) and spontaneous animal obese or hypertensive (related obesity) models for human diseases (SHR and Zucker rats). Therefore, we chose to study anti-obesity effects patterns within rats, finding a more selective anti-obesity effect in obese diseased rats than in the controls. In this extract experiment, however we attained mild anti-obesity (below 8%) data, this Dongchunghacho is not a diuretic or laxative. High doses of some anti-obesity foods are effective in suppressing fat accumulation in Zucker rats, but they are highly toxic to the testis over a 3-month period (Saito *et al.*, 2005). In a thirteen-week repeated oral toxicity study in Sprague-Dawely rats, mild reductions in body weight gain were observed dose-dependently in the *I. sinclairii* treated groups after 2 weeks. Interestingly, the weight of the abdominal adipose tissue surrounding the epididymides was greatly reduced by this Dongchunghacho; in parallel with a mild increase in body weight gain (Ahn *et al.*, 2004). It is possible that the cell membrane susceptibility, or the membrane receptor binding ability, is increased in disease- (hypertension or obese) oriented rats by the treatment of *I. sinclairii*.

It seems *I. sinclairii* might have a selective mode of action in hypertension-dependent obesity, showing better anti-obesity activity (SHR>Zucker>WKY>SD rats). Based on the extraction method, the hot water (above 90°C 1 hr boiling) extract had greater anti-obesity activity than the methanol (or ethanol) extract of *I. sinclairii*.

There are various anti-obesity drugs available, such as fat resumption inhibitors (inactivator of lipase) like, Orlistat (commercial name: Xenical) (Bray, 2001), which was approved by the FDA in 1999 for the long-term treatment of obesity. In this experiment, Xenical (30 mg/kg) treatments resulted in relatively weak anti-obesity effects compared to treatments of the *I. sinclairii* extract (hot water extract, 100 mg/kg) in Zucker rats (Fig. 1). However, the Xenical (100 mg/kg) treatment showed similar anti-obesity effects to the *I. sinclairii* hot water extract treatment (100 mg/kg) in the SD normal rats (Table 2).

There is a hypothesis that leptin cause diuresis/natriuresis (Jacksin and Herzer, 1999), and leptin, insulin, and growth hormone levels seem to regulate body composition, fat distribution, and fat mass (Molero-Conejo *et al.*, 2006). There were no significant differences in serum leptin concentrations among the Zucker obese rats treated with *Garcinia cambogia* over a 3-month period (Saito et al., 2005). On the other hand, Topiramate, an effective weight-loss inducer in in Zucker rats (Picard *et al.*, 2000), significantly increased leptin levels (7-9 days 100 mg/kg gavage dosing, 9.34% leptin increase). In our study, serum leptin concentration did no increase in the SD rats treated with *I. sinclairii*, but slightly increased in the Zucker rats over a 4-month period (data not shown). Further studies on the physiological role of *Isaria sinclairii* in obesity-related hypertension as well as its mechanism of action are required.

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