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Anti-diabetic effect of Cyclo-His-Pro (CHP)-enriched yeast hydrolysate in streptozotocin-induced diabetic mice

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The present study was designed to investigate the hypoglycemic effects of the daily oral dose of 0.50 to 0.75 g/kg of yeast hydrolysate (YH) containing high Cyclo-His-Pro (51.0 mg CHP/g YH) on normal and streptozotocin (STZ)-induced diabetic rats for 14 days. In STZ-induced diabetic rats, after administrations of the YH for 14 days, the body weight gain was significantly increased in dose dependent manner, and the plasma glucose levels were decreased approximately (60%) as compared to the STZ induced diabetic control group. Glucose level showed significant differences between the diabetic control (DC) and the YH administered groups in oral glucose tolerance test (OGTT) (P<0.05). Results of the OGTT showed a significant decrease in the area under curve (AUC) value of YH supplemented groups as compared to the DC group. The present data suggests that the CHP-enriched YH has potential anti-diabetic effect, which can help in the cure and management of diabetes.

Key words: Yeast hydrolysate, Cyclo-His-Pro (CHP), diabetes, streptozotocin.

INTRODUCTION

Diabetes mellitus (DM) is one of the major endocrine disorder and global public health problems, affecting nearly 10% of the world's population. DM consists of a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates and proteins; and the increased risk of complications from vascular disease (Davis and Granner, 2001). Conventional drugs used in its treatment are associated with drawbacks such as rigid and multiple dosing regimen, high-cost, inaccessibility and untoward effects. Scientific investigations of alternatives for diabetes have provided valuable clues for the development of therapeutic strategies. In the last few decades, there has been exponential growth in the field of alternatives by natural supplements, owing to their natural origin and lesser side effects (Yeh et al., 2003), since natural sources are usually considered to be less toxic with fewer side effects than synthetic sources (Pari and Umamaheswari, 2000). They have the potential to impart therapeutic effect in complicated disorders such as diabetes and its complications (Tiwari and Rao, 2002).

With more than 1000 strains of *Saccharomyces cerevisiae* categorized as generally recognized as safe, yeast is utilized in biochemical and medical applications (Carver, 1994) as well as many food industries such as brewing (Casey and Ingledew, 1983), winemaking (Shinohara et al., 2000) and baking (Chell, 1997). Several studies have demonstrated that yeast hydrolysate (YH) displays antiobesitic (Jung et al., 2009) and antidiabetic activity (Morley

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et al., 1981; Steiner et al., 1989). For these reasons, YH is receiving remarkable attention as a functional substance in the diet food market. The continuing development of functional foods is likely to entail increased use of different protein sources known to contain bioactive components.

Cyclo-His-Pro (CHP) is a naturally occurring cyclic dipeptide consisting of histidyl and proline and is a metabolite of thyrotropin-releasing hormone. As plasma levels of CHP in humans is increased after ingestion of glucose, CHP activity has been suggested to be related to glycemic control in patients with DM. Dietary feeding of CHP with zinc supplementation significantly improves insulin sensitivity and glucose clearance in diabetic animals and humans (Hwang et al., 2003; Song et al., 2001; Rosenthal et al., 2001). Furthermore, several studies have demonstrated that CHP decreases food intake, consequently, mimicking the action of leptin, the appetite control hormone (Morley et al., 1981; Steiner et al., 1989). Hence, CHP plays an important role in regulating insulin and leptin sensitivity, while no evidence of toxicity or side effects associated with its oral administration has been reported (Song et al., 2005, 2009).

We developed CHP containing high content of YH for possible applications of this cyclic dipeptide in metabolic disorder therapy. In the previous study (Jung et al., 2011), we increased the CHP content in the YH that was obtained from enzymatic hydrolysis and briefly assessed the antidiabetic effect by the oral glucose tolerance test (OGTT) in a type 1 diabetic animal model. The purpose of this study was to assess the anti-diabetic effect of YH with a high CHP content in normal and streptozotocin (STZ)-induced rats.

MATERIALS AND METHODS

Animals and diets

The animal protocol was approved by the Korea University Animal Care Committee. Male Sprague-Dawley (SD) rats, obtained at 6 weeks of age (200 ± 10 g), from Central Lab. Animal Inc. (Seoul, Korea) were housed individually in plastic cages with grated stainless steel floor. The colony was maintained at 24 ± 1°C with 60% relative humidity and a 12 h light/12 h dark cycle. The rats had *ad libitum* access to water and the rodent chow (Samyang Co., Seoul, Korea). Composition (g/100 kg of diet) of the chow was: moisture, 80; protein, 230; fat, 35; fiber, 50; carbohydrate, 600; and water.

Preparation of the diabetic rats

STZ (Sigma-Aldrich, St. Louis, MO, USA) was used to induce diabetes. The animals were given intraperitoneal injections of freshly prepared STZ (40 mg/kg in 0.01 M citrate buffer, pH 4.5) for 5 days, and the normal control (NC) group was injected with 0.01 M citrate buffer only. Five days after STZ treatment, blood was collec-ted from the tip of the tail vein, and fasting glucose level was measured (Takamura et al., 1999). The STZ injection destroyed a sufficient number of islet beta cells to be a model of type 1 diabetes with the glucose level of over 300 mg/dl. After the STZ-induction period, the rats were separated into four groups (six rats/group) based on their blood glucose level and body weight. Treatment was started 8 days after the STZ injection.

YH preparation

An 8% yeast suspension was hydrolyzed for 48 h using Flavourzyme (endoprotease and exopeptidase from *Aspergillus oryzae*). The hydrolysis temperature was 50°C for the crude enzymes, and the enzyme : yeast substrate ratio was 1:100 for the enzymes. The hydrolysis of yeast with enzymes was performed in 0.01 M phosphate buffer. pH was adjusted to the optimal values for the Flavourzyme specific proteases (pH 7.0) before hydrolysis was initiated. Hydrolysis was inactivated by heating at 90°C for 5 min. The YH obtained from enzymatic hydrolysis was first passed through a 0.2 μm membrane filter (Satocon cassette, Sartorius, Goettingen, Germany). A portion of the solution was removed immediately, and the filtrate was then pumped through a 10 kDa molecular weight cut-off membrane (Satocon cassette, Sartorius). The YH obtained from ultrafiltration was adsorbed with active carbon. The YH obtained from ultrafiltration, and the resulting substance were dried and used as the YH (51.0 mg/g CHP).

YH administration

YH was suspended in distilled water and administered orally through an intragastric tube at doses of 0.50 or 0.75 g/kg body weight. The volume of administrated extract was 1 ml for each animal. The rats were divided into four groups (eight rats/group): NC: 1 ml water for normal control; DC: 1 ml water for diabetic rats; YH-1: 0.50 g/kg body weight YH; or YH-2: 0.75g/kg body weight YH for diabetic rats, and each group of rats was treated daily for 14 days using an intragastric tube.

At the end of the experimental period, the rats were anesthetized with ethyl ether, and blood was collected from the abdominal artery into a heparinized sterile tube. Plasma was obtained by centrifugation at 1,800 × *g* for 30 min and stored at -80°C until further analysis. The liver, spleen and kidneys were excised and weighed after sacrificing the animals.

Fasting blood glucose level

Blood glucose levels were monitored every week after a 12 h fast in venous blood taken from the tail vein using a glucose analyzer. Fasting blood glucose was measured after 14 days of YH treatment during which the animals were fed a normal diet. Rats were fasted for 12 h, and blood was collected from the tip of the tail vein. Blood glucose level was measured using a blood glucose analyzer (Superglucocard II, Arkray Inc., Kyoto, Japan) based on the glucose oxidase method (Barham and Trinder, 1972). Results are expressed as glucose mg/dl blood.

Oral glucose tolerance test

On the day of animal sacrifice, after an overnight fast, 0 min blood was taken from the tip of the tail vein from all the rats. Rats that had been administered YH (0.75 g/kg body weight), received oral load of 30% glucose solution (2 g glucose/kg body weight). Blood samples were collected from the tail vein at 30, 60, 90 and 120 min after the oral glucose load and treated as described previously for the plasma glucose analysis (Han et al., 2007). Blood glucose level was expressed as increments from baseline. Incremental areas under the response curves (AUC) were calculated using the trapezoidal rule, with fasting levels considered as baseline.

Statistical analysis

All statistical analyses were performed using the Statistical Package

Table 1. Effect of yeast hydrolysate on body weight in normal and STZ-induced diabetic rats.

Group[1]	Initial body weight (g)	Final body weight (g)	Body weight gain (g/14 days)
Normal control	211.20 ± 1.02[2a]	236.50 ± 3.96[a]	25.30 ± 2.94[a]
Diabetic control	195.80 ± 1.39[b]	169.30 ± 2.69[c]	-26.50 ± 1.31[d]
Diabetic YH-1	192.10 ± 0.65[b]	179.80 ± 0.94[c]	-12.30 ± 0.29[c]
Diabetic YH-2	195.80 ± 1.22[b]	197.00 ± 5.88[b]	8.40 ± 2.56[b]

[1]Diabetic YH-1; STZ-induced diabetic rats given 0.50 g/kg of yeast hydrolysate; Diabetic YH-2, STZ-induced diabetic rats given 0.75 g/kg of yeast hydrolysate. [2]Mean ± SEM; values with different superscripts letters within the same column are significantly different at $P<0.05$ by Duncan's multiple range test.

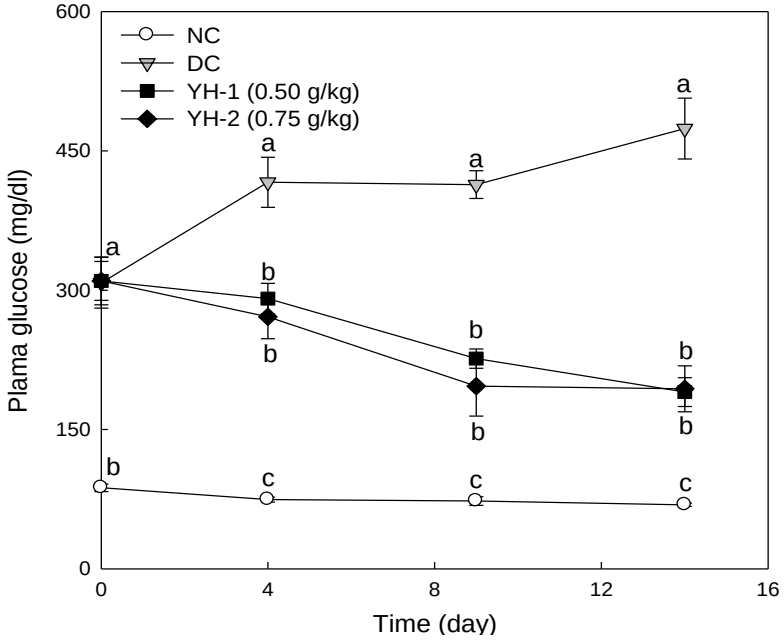

Figure 1. Effect of yeast hydrolysate on plasma glucose in normal and STZ-induced diabetic rats. Values with different superscripts letters within the column are significantly different at $P<0.05$ by Duncan's multiple range test; NC, 1 ml water for normal control; DC, 1 ml water for diabetic rats; YH-1, 0.50 g/kg body weight YH; YH-2, 0.75 g/kg body weight YH for diabetic rats.

for Social Sciences (SPSS) version 12.0 (SPSS Inc., USA). The differences among groups were evaluated by one-way analysis of variance (ANOVA) and Duncan's multiple range tests. All data were reported as the mean and standard error. A level of $P<0.05$ was used as the criterion for statistical significance.

RESULTS

Body weight gain in STZ-induced mice during the 14 day period

YH or vehicle was administered orally to STZ-induced mice for 14 days, and body weight was measured. As shown in Table 1, no significant differences in initial body weight were observed among the STZ-diabetic groups. The NC group continued to gain weight until the end of the study. STZ produced significant body weight losses as compared to that in the NC group. After 14 days of testing, final body weights and mean body weight gains were significantly lower ($P<0.05$) in all STZ-diabetic groups as compared to those of the NC group. Mean weight gains in the YH administered groups were significantly higher ($P<0.05$) than those in the DC group, and significant improvements ($P<0.05$) in body weight were observed in the YH-2 group.

Fasting blood glucose levels in STZ-induced mice during the 14 day period

The effect of repeated oral administration of YH or vehicle on fasting blood glucose levels in the STZ-diabetic groups during 14 days of treatment are presented in Figure 1. Intraperitoneal administration of STZ to rats

Table 2. Effect of yeast hydrolysate on organ weights in normal and STZ-induced diabetic rats.

Group[1]	Liver (g/100 g of body weight)	Spleen (g/100 g of body weight)	Kidney (g/100 g of body weight)
Normal control	4.42 ± 0.10[2b]	0.57 ± 0.02[NS]	0.48 ± 0.04[NS]
Diabetic control	4.92 ± 0.10[a]	0.60 ± 0.03	0.60 ± 0.06
Diabetic YH-1	4.52 ± 0.10[ab]	0.58 ± 0.03	0.54 ± 0.04
Diabetic YH-2	4.42 ± 0.01[b]	0.59 ± 0.05	0.51 ± 0.05

[1]Diabetic YH-1, STZ-induced diabetic rats given 0.50 g/kg of yeast hydrolysate; Diabetic YH-2, STZ-induced diabetic rats given 0.75 g/kg of yeast hydrolysate. [2]Mean ± SEM; values with different superscripts letters within the same column are significantly different at $P<0.05$ by Duncan's multiple range test; NS, not significant.

caused a significant diabetogenic response with significant increases ($P<0.05$) in fasting blood glucose levels as compared to those in the NC group. Plasma glucose level increased continuously in the DC group during the experimental period, reaching a final level of approximately 500 mg/dl. YH administered at two different doses of 0.50 and 0.75 g/kg body weight to the STZ-treated diabetic groups resulted in a significant reduction ($P<0.05$) of fasting blood glucose levels, which was associated with treatment duration. After 14 days of treatment, the fasting blood glucose levels in the STZ-induced diabetic mice treated with YH at 0.75 g/kg (193.80 ± 10.12 mg/dl) were significantly lower ($P<0.05$) as com-pared to those of the DC group (474.00 ± 13.35 mg/dl).

Organ weights in the STZ-induced mice

We investigated the influence of YH on the liver, spleen and kidney weight (Table 2). The DC group (4.92 ± 0.10 g/100 g body weight) had significantly greater ($P<0.05$) liver weights than the NC group (4.42 ± 0.10 g/100 g body weight). The liver weight was significantly changed by YH administration in a dose dependent manner. Spleen weight was not significantly different ($P<0.05$) regardless of diabetes induction. However, the DC group (0.60 ± 0.03 g/100 g body weight) exhibited greater spleen weight than the NC (0.57 ± 0.2 g/100 g body weight) and YH administered groups (0.58 ± 0.03 and 0.59 ± 0.05 g/100 g body weight, respectively). Diabetes induced by STZ injection resulted in increased kidney weight; the YH administered groups had decreased kidney weight when compared with the DC group.

Oral glucose tolerance test

The OGTT and the calculated AUC were used to determine the glucose response to YH administration. As a result, the DC, YH-1, YH-2 and NC groups showed significant increases ($P<0.05$) in blood glucose levels at 30 min after approximately 522.00 ± 6.21, 443.00 ± 3.59, 431.50 ± 6.29 and 209.30 ± 3.10 mg/dl glucose gavages, respectively. However, the DC group (248.80 ± 7.68 mg/dl) did not recover baseline glucose level even after 120 min. The OGTT results show that blood glucose decreased significantly ($P<0.05$) in the YH administered groups when compared with that in the DC group (Figure 2A). Furthermore, the AUC was significantly smaller in the YH administered groups than in the DC group (Figure 2B). The AUC of the STZ-induced diabetic mice treated with YH-2 was significantly smaller ($P<0.05$) than that in the YH-1 group.

DISCUSSION

DM is a chronic metabolic disorder affecting a major proportion of the population worldwide. Conventional therapies for DM have many disadvantages such as side effects and a high rate of secondary failure. In contrast, natural sources are expected to have similar efficacy without side effects as those of conventional drugs. CHP exists in several common nutritional supplements, and it is known to have antiobesity effects (Jung et al., 2009) as well as antidiabetic activity (Morley et al., 1981; Steiner et al., 1989). This study was undertaken to evaluate the hypoglycemic activity of YH with a high content of CHP in normal and STZ-induced diabetic rats.

STZ is used to induce DM by selectively affecting pancreatic β-cells. Thus, STZ affects endogenous insulin release and increases blood glucose levels (So et al., 2011). Final body weights and mean body weight gains were significantly lower ($P<0.05$) in all STZ-diabetic groups after 14 days of testing, as compared to those of the NC group (Table 1). The induction of DM with STZ is associated with a characteristic loss of body weight due to increased muscle loss (Chatterjee and Shinde, 1994). However, hypoglycemic treatment with medicinal herbs may help STZ-induced diabetic rats to recover from hyperglycemia (Hwang et al., 2005; Prasad et al., 2009). Treatment with YH significantly improved body weight, indicating that it prevented muscle loss due to hyperglycemic conditions.

The liver weights were significantly greater ($P<0.05$) in the DC group as compared to those of the NC group, and the YH administered groups showed significant changes in liver weight in a dose-dependent manner as compared to those of of the DC group. The lipid metabolic pathway is activated by acetyl-CoA in STZ-induced diabetic rats, rather than normal glucose metabolism, due to reduced

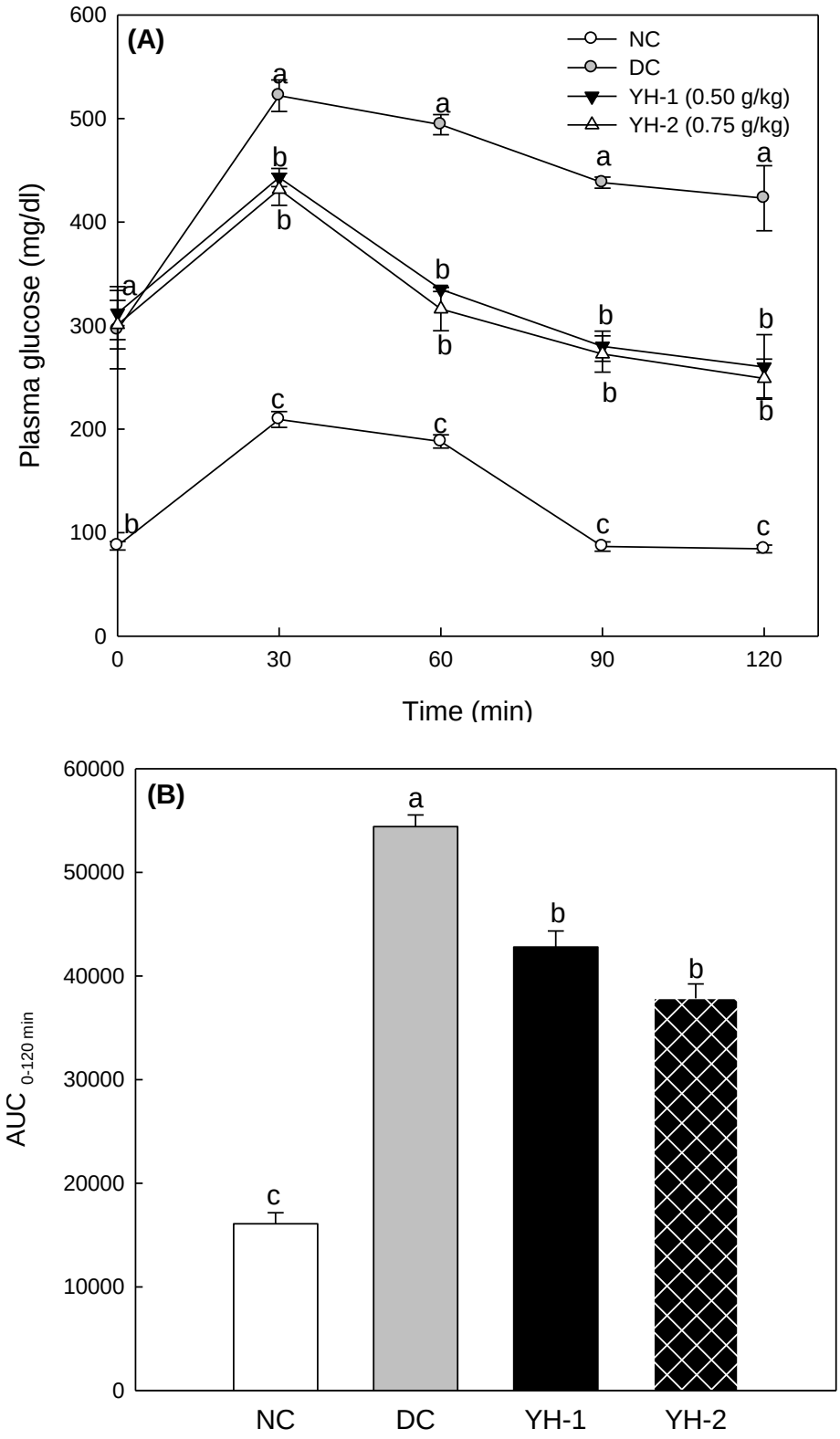

Figure 2. Effect of yeast hydrolysate on oral glucose tolerance test (A) OGTT and integrated area under the curves (AUCs) over a period of 0 to 120 min (B) in normal and STZ-induced diabetic rats). Values with different superscripts letters within the column are significantly different at $P<0.05$ by Duncan's multiple range test. NC, 1 ml water for normal control; DC, 1 ml water for diabetic rats; YH-1, 0.50 g/kg body weight YH; YH-2, 0.75 g/kg body weight YH for diabetic rats.

insulin levels (Goldstein and Brown, 1977). Based on this hypothesis, increased synthesis and accumulation of heaptic triglycerides can be well explained by the liver hypertrophy in STZ-induced diabetic rats. DM induced by STZ resulted in increased kidney weight. The DC group exhibited greater kidney weight gains than the NC group. However, the YH administrated groups showed decreased kidney weights as compared to the DC group, indicating that renal enlargement commonly occurs in DM patients. The increase in kidney volume and size may be due to the increased glomerular filtration rate in early stage DM. McNeill (1999) reported that such diabetic animals develop glomerular hypertrophy, renal hyper filtration and enlargement, increased urinary albumin excretion, and glomerular extracellular matrix accumu- lation within weeks of DM onset. The results of this study support the possibility that CHP in YH might be effective in preventing renal failure in diabetic animals.

The intraperitoneal administration of STZ to SD rats caused a significant diabetogenic response with significant increases in the levels of fasting blood glucose as compared to those of the NC group. After 14 days of treatment, the fasting blood glucose levels of the STZ-induced diabetic mice treated with YH at 0.75 g/kg were significantly lower (P<0.05) than those of the DC group. As a result, *in vivo* treatment with YH resulted in a significant reduction in blood glucose level due to an increased rate of glucose elimination. The OGTT results reveal that blood glucose level was significantly decreased in the YH administered groups as compared to that of the DC group. Furthermore, the AUC was significantly lower in a dose-dependent manner (P<0.05) in the YH administered groups as compared to the DC group. Hyperglycemia was induced by intraperitoneal STZ. Maintaining blood glucose at levels close to normal and preventing diabetic complications are major goals in the treatment of DM (Control and Group, 1994). YH may enhance glucose utilization, because it significantly decreases blood glucose levels in glucose-loaded rats, although the exact mechanisms are unclear.

Similar results were reported by Song et al. (2001), in which a prostate extract containing zinc and CHP significantly decreased blood glucose and improved glucose tolerance and insulin sensitivity in STZ-induced diabetic rats. These results indicate that CHP has a strong ability to stimulate intestinal zinc absorption, cellular zinc uptake and promoted glucose utilization (Rosenthal et al., 2001; Song et al., 2001, 2003). Zinc deficiency critically affects DM, because zinc activates insulin receptor β-subunits, thereby exerting an influence on glucose metabolism (Song et al., 2001). Although, the exact mechanism of the antidiabetic effect of CHP has not been clearly established, it is likely that CHP is involved in regulating insulin and leptin sensitivity by stimulating zinc metabolism because of the following arguments: oral intake of CHP plus zinc significantly improves glucose tolerance in diabetic and overweight animals in the state of decreased or unchanged insulin levels (Hwang et al., 2003; Song et al., 2001); high CHP concentrations inhibit insulin and glucagon secretion from islet cells *in vitro* (Wilber et al., 1984); and CHP stimulates zinc transport mechanisms across the small intestine and muscle cell membrane to increase zinc use (Rosenthal et al., 2001). Accordingly, it is assumed that the YH-containing CHP might affect zinc metabolism.

In conclusion, YH containing CHP was useful as a therapeutic agent to improve glucose tolerance in diabetic rats. Further investigations are needed to elucidate the molecular mechanisms by which the antidiabetic effects of CHP are mediated.

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