Research Note

Effect of Yeonsan Ogye bioactive peptides on anti-oxidant indexes in rats' liver*

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ABSTRACT

Purpose: This study investigated the effect of bioactive Yeonsan Ogye peptides (YOPs) intake on changes in the hepatic anti-oxidant indexes in male rats. Methods: Sprague-Dawley male rats were divided into 3 groups and given a casein-based AIN-93G diet and distilled water *ad libitum* without any added YOPs (control), distilled water with 250 mg of YOPs (Y250), or 500 mg of YOPs (Y500) per kg of body weight for 4 weeks. YOP dose was decided as referred to in the referenced study where toxicity did not occur. The hepatic anti-oxidant indexes were determined using a commercial kit. Statistical analysis was performed using SPSS version 23.0 and are expressed as mean ± standard error of mean. Differences among the groups were evaluated by one-way analysis of variance followed by post hoc Duncan's multiple comparisons test. Results: There were no differences in the body weights, weight gain, food intake, food efficiency ratio, or organ weight, including liver, kidney, spleen, thymus, and epididymal fat, among all of the groups. The hepatic nitric oxide (NO) level in the Y500 group was lower than that in the control and Y250 groups, and the hepatic malondialdehyde (MDA) level was lower in the Y500 group than in the Y250 group. The differences in hepatic superoxide dismutase (SOD) and catalase (CAT) activities were not statistically significant between the groups. From these results we speculated that YOPs may have anti-oxidative abilities to regulate NO and MDA production without affecting SOD and CAT activities. Conclusion: YOPs are presumed to act as anti-oxidants in the animal or human body.

KEY WORDS: anti-oxidants, bioactive peptides, liver, Sprague-Dawley rats, Yeonsan Ogye

Introduction

Korea has a long history of traditional oriental medicine whose materials are derived from nature. Yeonsan Ogye (YO) is one of Korea's native poultry species which has many positive effects such as prevention of anemia, coronary heart disease, and treatment of deep-seated sore [1,2]. Recently, Kim et al. have reported that YO extracts have various biological activities including anti-inflammation, anti-oxidation, and immuno-enhancement in cell line [3]. Bioactive peptides are generally defined as a small peptide with hormone or drug-like physiological activity and many animal and plant food proteins are used as sources of bioactive peptides [4,5]. A study using the bioactive peptides of Ogye was performed *in vitro*; suggested that Ogye peptides have anti-oxidative effects [6]. However, the anti-oxidant capacity of bioactive Yeonsan Ogye peptides (YOPs), which has potential for use as an anti-oxidant functional food, has not been studied yet in animal model.

Therefore, in this study, we have investigated the change of hepatic anti-oxidant index levels of three-week-old Sprague-Dawley male rats depending on the amount of YOPs to their feed.

Methods

Animals and diets

This study was approved by the Institutional Animal Care and Use Committee of Daejeon University (Approval No. DJUARB2016-025). Three-week-old Sprague-Dawley male rats were purchased from BioBridge (Seoul, Korea).

YOPs was produced in the laboratory of Joongbu University [7] and a dose of YOPs was decided to be mixed to 250 and 500 mg/kg of body weight as referred to in the study of Kim et al. where toxicity did not occur [3]. After a 4-day acclimation period with non-purified diet (BioBridge, Seoul, Korea), rats were divided into 3 groups and given a casein-based AIN-93G diet and distilled water *ad libitum* without any added YOPs (control), distilled water with 250 mg of YOPs (Y250), or 500 mg of YOPs (Y500) per kg of body weight for 4 weeks.

The distilled water with YOPs was fed through oral gavage once per day using flexible zonde needle (Natsume Seisakusho Co., Ltd., Osaka, Japan). The body weight and food intake of rats were recorded once a week, periodically. At 4 weeks, rats were fasting 12 hours and killed by exsanguinations under ether anesthesia. Blood was collected and plasma was separated, while liver, kidney, spleen, thymus, and epididymal fat were removed and stored at -80°C immediately.

The determination of hepatic anti-oxidant index levels

The level or activity of hepatic anti-oxidant indexes in this study was determined using a commercial kit. The hepatic nitric oxide (NO) level was measured by Nitric Oxide Colorimetric Assay Kit (Cat No. K262-200, BioVision, Milpitas, California, USA) and the hepatic malondialdehyde (MDA) level was measured by Spect-

rophotometric Assay for Malondialdehyde (Cat No. 21044, OxisResearch™, FosterCity, CA, USA). The hepatic activities of superoxide dismutase (SOD) and catalase (CAT) were measured by Superoxide Dismutase Assay Kit (Cat. 706002, Cayman Chemical, Ann Arbor, MI, USA) and Catalase Assay Kit (Cat No. 707002, Cayman Chemical, Ann Arbor, MI, USA), respectively.

Statistical analysis

Data were analyzed with SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and are expressed as mean ± standard error of mean. Differences among the 3 groups were evaluated by one-way analysis of variance followed by post hoc Duncan's multiple comparisons test. A p-value < 0.05 was considered as statistically significant.

Results and Discussion

There were no differences in the initial and final body weights, weight gain, food intake, food efficiency ratio, and organ weight at day killed including liver, kidney, spleen, thymus, and epididymal fat among all of the groups (Table 1).

To confirm whether the YOPs leads to the regulation of anti-oxidative reaction, we measured the level of NO and MDA, and the activity of SOD and CAT which is known as anti-oxidant indexes in a rat's liver. The measurement of MDA is widely used as an indicator of

Table 1. Body weight, weight gain, food intake, food efficiency ratio, and organ weight in the control and experimental Sprague-Dawley male rats

	Control[1]	Y250[2]	Y500	p-value
Body weight (g)				
Initial (g)	55.19 ± 2.72	57.19 ± 4.67	56.94 ± 4.57	NS[3]
Final (g)	249.69 ± 22.97	235.63 ± 31.39	240.31 ± 14.79	NS
Weight gain (g/day)	7.55 ± 0.92	6.89 ± 1.27	7.19 ± 0.58	NS
Food intake (g/day)	17.60 ± 1.37	17.32 ± 2.26	16.95 ± 1.01	NS
Food efficiency ratio (%)[4]	42.87 ± 3.68	39.59 ± 2.63	42.39 ± 1.27	NS
Organ weight (g) at day killed				
Liver	8.35 ± 1.16	8.02 ± 1.58	8.01 ± 0.77	NS
Kidney	1.04 ± 0.08	0.97 ± 0.19	0.96 ± 0.06	NS
Spleen	0.65 ± 0.08	0.64 ± 0.14	0.67 ± 0.06	NS
Thymus	2.18 ± 0.68	1.92 ± 0.55	1.99 ± 0.53	NS
Epididymal fat	0.90 ± 0.17	0.85 ± 0.12	0.93 ± 0.10	NS

1) Values are expressed as means ± SEM (n = 9 per group). Significant differences among 3 groups were analyzed by one-way ANOVA followed by post hoc Duncan's multiple comparisons test.
2) Y250, fed with distilled water + YOPs containing 250 mg/kg of body weight; Y500, fed with distilled water + YOPs containing 500 mg/kg of body weight
3) NS means no significant difference among the 3 groups.
4) Food efficiency ratio = ([final weight - initial weight]/28 days food intake) × 100
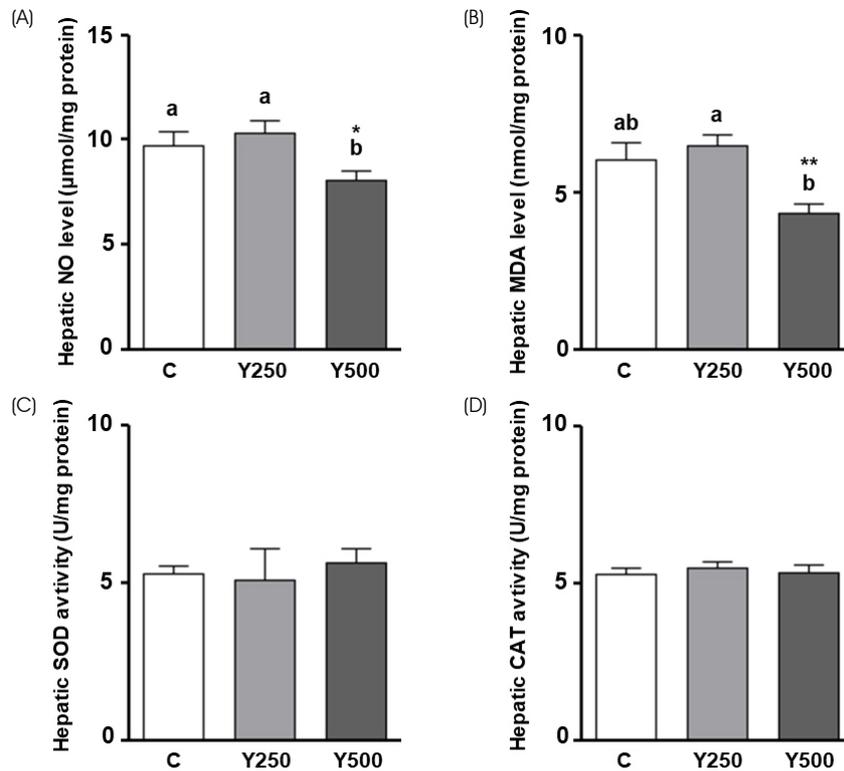
Fig. 1. Hepatic level of (A) NO and (B) MDA and hepatic activity of (C) SOD and (D) CAT of rats fed with experimental diets for 4 weeks. C, control group; Y250, fed with distilled water + YOPs containing 250 mg/kg of body weight; Y500, fed with distilled water + YOPs containing 500 mg/kg of body weight. Values are expressed as means (n = 9 per group). Significant differences among 3 groups were analyzed by one-way ANOVA followed by post hoc Duncan's multiple comparisons test.

oxidative stress, and SOD and CAT denotes anti-oxidant enzymes [8]. During the inflammatory process, NO and superoxide are produced from immune cells, and these may react together producing oxidative active molecule, which can cause lipid oxidation [9].

Fig. 1 indicated the hepatic level of NO and MDA and the hepatic activity of SOD and CAT of 3 groups (Fig. 1). The hepatic NO level in the Y500 (8.36 ± 0.38 μmol/mg protein) group was significantly lower than that in the control (9.71 ± 0.66 μmol/mg protein) and Y250 (10.34 ± 0.57 μmol/mg protein) groups ($p < 0.05$, Fig. 1A). As Compared with in the Y250 (6.46 ± 0.37 nmol/mg protein) group, the hepatic MDA level was decreased in the Y500 (4.33 ± 0.28 nmol/mg) group ($p < 0.01$, Fig. 1B). These results were partially different from the study that treated the extract of YO in rat's macrophage, showing that the levels of NO and cytokine were decreased whereas there was no difference in dose of extracts [3]. And our results were similar with other research which evaluated the effect of bovine lactoferricin to inhibition of inducible NO synthase mRNA expression in human articular chondrocyte

[10]. Moreover, the result of MDA was also similar to other study when investigating the effect of bioactive peptide on the level of MDA. Carnosine known as bioactive peptide in some species of vertebrates leads to the reduction of hepatic MDA level in rats [11]. Although few studies that using YOPs were conducted, we can consider that YOPs may have abilities to regulate NO and MDA production, and may have the effectiveness is higher as YOPs dose is higher.

The statistical significance of hepatic SOD and CAT activities which concern as anti-oxidant enzymes among the groups did not occur in this study (Fig. 1C and 1D). According to oxidative stress pathway, anti-oxidative effect on free radical removal could explain in two ways; first is that promoting the activities of anti-oxidant enzymes, and second is that anti-oxidants themselves removing free radicals [12].

Therefore we speculate that YOPs seems to have a role as an anti-oxidant itself. Moreover, one may speculate two possibilities about these results. First, toxic products known as free radicals may not be enough generated in

liver to increase the activity of SOD and CAT, because we used healthy animal models. Second, the reduction of NO and MDA may be attributed to other mechanisms unrelated to those of the anti-oxidants. To investigate these possibilities, experiments should be added to whether or not hydrogen peroxide, hydroxyl radicals, and superoxide radicals should be produced.

According to these results, we conclude that YOPs can cause hepatic anti-oxidation. The strength of this study is being the first to demonstrate YOPs' efficacy. This study is worthwhile based on the results for further researches of YOPs, and it is also meaningful in contributing to the development of functional foods with YOPs which may have anti-oxidative effects. In this study, we experimented with male rats to exclude the effects of hormonal changes according to menstrual cycle in female rats. However further study using both gender rats should be conducted as a preclinical study to examine the efficacy of YOPs.

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