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Antioxidant Activity of Ethanol and Water Extracts from Lentil (*Lens Culinaris*)

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Abstract The purpose of this study was to evaluate the antioxidative activities of water and ethanolic extracts from lentil (*Lens Culinaris*). The antioxidant effects of lentil extracts were evaluated, including 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity and ferric reducing antioxidant power (FRAP). We also evaluated the protection effect on hydrogen peroxide-induced oxidative damage in PC-12 cells via propidium iodide (PI) staining using a flow cytometer. Lentil extracts decreased cell death in PC-12 cells due to hydrogen peroxide-induced oxidative damage in a dose-dependent manner. The findings of this study suggested that lentil has the potential to protect against hydrogen peroxide-induced cell damage and should be considered as a prospective functional food.

Keywords: *Lentil, antioxidant, neuro-protection, Cell apoptosis*

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1. Introduction

Plants are one of the most important sources of medicine [1], and their secondary metabolites are leading source of therapeutic agents [2]. Wide diversity of antioxidants has been obtained from herbal resources which have different physical and chemical composition, properties and mechanism [3]. Lentil (*Lens culinaris*) is an ancient crop of classical Mediterranean civilization and continues to play an important role in human health [4]. Also, lentil seeds are bioactive dietary supplements and as an antioxidant protect the human body from free radicals as well as retard the progress of many chronic diseases including hypertension, diabetes mellitus, cardiovascular diseases and cancer [5].

Antioxidants are substances capable of inhibiting the oxidation, reducing the concentration of free radicals in the body and/or chelating metal ions, preventing the lipid peroxidation [6]. As natural dietary antioxidants, phenolic compounds from plants may protect cell membranes against damage mediated by oxygen radicals [7]. Research on the effects of dietary polyphenols on human health has developed considerably. It strongly supports a role for polyphenols in the prevention of degenerative diseases, particularly cardiovascular diseases and cancers [7]. Currently, researches show that rich sources of antioxidants are important for the maintenance of human health, and increased attention has been given to these compounds found in plants. This is due to epidemiological evidence that have shown that regular consumption of vegetables is associated with reduced mortality and morbidity from some chronic diseases.

The purpose of the present study is to investigate antioxidant activity and neuro-protective effects against oxidative damage of extract from lentil.

2. Materials and Methods

2.1. Materials

The lentil was obtained at local maket (Seoul, Korea). 3-[4,5-dimethy-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay kit were procured from Sigma Aldrich (St. Louis, MO, USA). Culture plates and culture dishes were purchased from Nunc, Inc. (North Aurora Road, IL, USA). Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Hyclone (Logan, UT, USA). All other reagents were of the highest grade available commercially.

2.2. Sample Preparation

Antioxidant activities were extracted from lentils following a slightly modified of the method described by Zhang et al. [8]. The 70% ethanolic extracts were prepared by three times with 70% ethanol under reflux for 24 h and filtered at room temperature (RT). The filtrate was evaporated by an evaporator (EYELA, Tokyo, Japan) at 45°C and then lyophilized in a freeze-dryer to obtain freeze-dried extract. In addition, the water extracts were decocted for approximately 6 h three time at 70°C, and the filtrate was evaporated by an evaporator at 55°C and then lyophilized in a freeze-dryer to obtain freeze-dried extract.

2.3. ABTS radical Scavenging Activity

The total antioxidant activities of extracts from lentil were measured by the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay involving the preformed ABTS radical cation [9]. ABTS radical cation was produced by the 7 mM ABTS

stock solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at RT for 14 h before use. To determine the scavenging activity, 0.9 mL of ABTS reagent was mixed with 0.1 mL of extracts and the absorbance was measured at 734 nm after 6 min of reaction at RT. The antioxidant activities of lentil extracts were expressed by trolox equivalents antioxidant capacity (TEAC), as mM trolox equivalents/mg extract.

2.4. FRAP Assay

The FRAP (ferric reducing antioxidant power) method was conducted according to Benzie and Strain [10]. To conduct the assay, a 3 mL aliquot of a FRAP reagent, a mixture of 0.3 M acetate buffer, 10 mM TPTZ in 40 mM HCl, and 20 mM ferric chloride, were combined with 1 mL of lentil extract. To determine the antioxidant capacity of the samples, the absorbance values were compared with those obtained from the standard curves of $FeSO_4$ (0 ~ 10 mM). The antioxidant capacity values were expressed as mM $FeSO_4$ equivalent in mg extract (mM $FeSO_4$ eq./mg extract).

2.5. Cell Culture

PC-12 cell line obtained from American Type Culture Collection (ATCC, Rockville, MD, USA), were cultured in DMEM supplemented with penicillin, streptomycin and 10% heat-inactivated FBS in 5% CO_2, 95% air and humidified atmosphere at 37°C.

2.6. MTT Assay

For evaluating the cytotoxicity of extract from lentil, the cells were harvested using phosphate buffered saline (PBS) containing 0.15% trypsin and 0.08% EDTA, Cells were incubated in well plates at a density of 5 x 10^5 cells/well. MTT solution was added to each well. Following incubation for 4 h at 37°C in 5% CO_2, the supernatant was removed. The medium was removed and the cells were washed by PBS twice, and the formazan crystals produced in viable cells were solubilized in 200 µL DMSO. The absorbance was measured using a microplate reader (Tecan Trading AG, Männedorf, Switzerland) at 550 nm. All experiments were performed with three replicates [11].

2.7. Measurement of Cell Apoptosis

For sub-G1 and cell cycle analysis, cells were suspended in ethanol with 0.5% Tween-20 and left for 24 h at 4°C. The cells were then harvested by centrifugation and resuspended in 1.0 mL of phosphate-buffered saline containing 0.05 mg/mL of propidium iodide (PI) and 10 µg/mL of RNase A, and incubated at 37°C for 30 min. Analysis of apoptotic cell death was performed by measuring the hypodiploid DNA contents using a flow cytometer (FACS-Caliber; Becton Dickinson; Franklin Lakes, NJ, USA). The cells in the sub-G1 population were considered apoptotic cells, and the percentage of cells in each phase of the cell cycle was determined [12].

2.8. Statistical Analysis

Statistical analyses were performed 3 times for all the experiments. The data are expressed as the mean ± one standard error of mean (SEM). Statistical analyses were assessed by Student's *t*-test for paired data. Graph Pad Prism software version 4.00 (Graph Pad Software Inc., San Diego, CA) was used. Significant differences ($p < 0.05$) between the mean values of the triplicate samples were determined for various assays.

3. Results and Discussion

The ABTS radicals was shown that the scavenging activity of ethanol and water extracts evidenced 0.936 ± 0.03 and 1.011 ± 0.02, respectively. Also, antioxidant capacities results showed ethanol extracts have a higher FRAP antioxidant activity than water extracts, values were 1.662 ± 0.02 and 1.539 ± 0.04, respectively (Table 1). The effect of lentil extracts that the viability of PC-12 cells were examined by MTT assay. PC-12 cells were treated with ethanol and water extracts from lentil at various concentrations (0, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL, respectively) for 18 h. As shown in Figure 1 (A, B), cell viability was not reduced by lentil extracts in PC-12 cells proliferation. Cell apoptosis analysis showed the distribution of apoptotic cells throughout the cell cycle, as shown in Figure 2 (A, B), where the percentage of apoptotic cells in non-treated cells was 5.21%. The percentage of apoptotic cells was observed 32.62% at 1.0 mM oxidative damage and the ethanol extracts treated cells were 26.02, 24.23, 19.11 and 14.52% at 0.125, 0.25, 0.5 and 1.0 mg/mL, respectively. In addition, water extracts treated cells were 23.12, 20.30, 16.15 and 11.67% at 0.125, 0.25, 0.5 and 1.0 mg/mL, respectively. Therefore, the lentil extracts protect neuronal cells against oxidative damage. The early reported that the fermentation of lentils is an eligible process to obtain water soluble extracts with potential antihypertensive compounds (GABA and ACE inhibitors) as well as antioxidant properties [13]. Many previous studies have shown that the food compounds effect of biological antivities *in vitro* and *in vivo*, but only a little researches of lentil on antioxidant property were reported. In the present study, we showed that antioxidant and neuro-protective effects in PC-12 cells from lentil extracts. Furthermore, the regulatory effect of lentil extracts in molecular mechanisms in animal will be needed.

Table 1. Values for ABTS radical scavenging and FRAP from the lentil extracts.

Sample	TEAC (extract of mM trolox eq./mg)[a]	FRAP (extract of mM $FeSO_4$ eq./mg)[a]
Ethanol extract	0.936 ± 0.03	1.662 ± 0.02
Water extract	1.011 ± 0.02	1.539 ± 0.04

[a]Values represent means ± SD (n = 3).

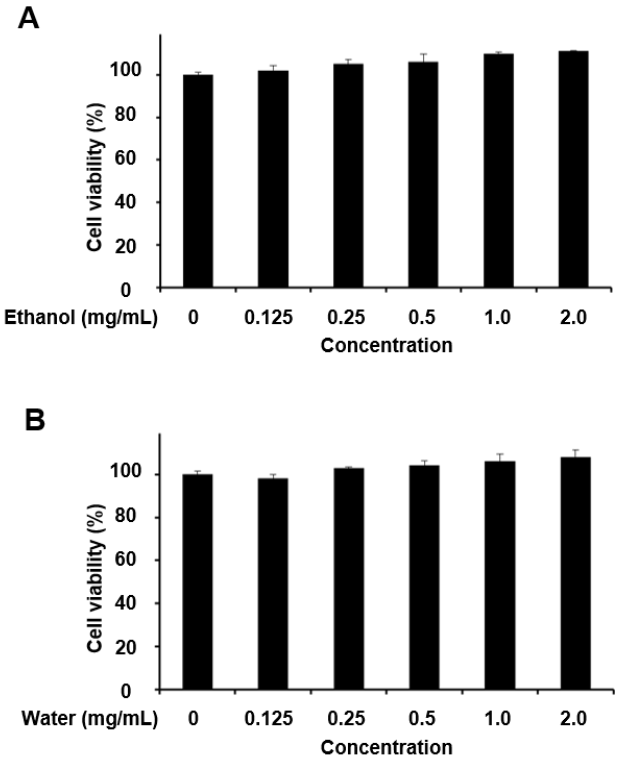

Figure 1. Effect of lentil on the cell viability of ethanol extract (A), and water extract (B) cells using the MTT assay. various cells were incubated with 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL for 18 h. The results are shown as percentages of control samples. Data are presented as the mean ± S.E.M. (n = 3) for three independent experiments. Significance was determined by Student's *t*-test. $p < 0.05$

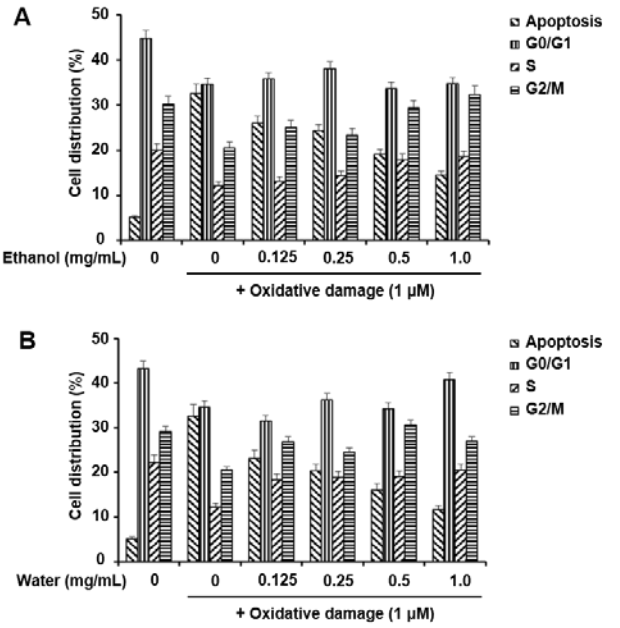

Figure 2. Cell death and cycle of ethanol extract (A), and water extract (B) using flow cytometry analysis. various cells were incubated with 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL of extracts for 24 h. The results are shown as percentages of control samples. Data are presented as the mean ± S.E.M. (n = 3) for three independent experiments. Significance was determined by Student's *t*-test. #$p < 0.05$

4. Conclusions

The lentil ethanolic extract had higher levels of some antioxidant activities compared to water extract. However, both ethanol and water offered protection in PC-12 cells against oxidative damage. In this regard, molecular mechanisms by which lentil extracts induced antioxidant enzymes need to be evaluated.

References

[1] Yesilada, E., Ustun, O., Sezik, E., Takaish, Y., Ono, Y., and Honda, G., "Inhibitory effect of Turkish folk remedies on inflammatory cytokines: Interleukin-1-alpha, interleukin-1-beta and tumor necrosis factor-alpha" J Ethnopharmacol 58(1), 59-73. 1997.

[2] Caragay, A.B., "Cancer-preventive foods and ingredients" Food Technol 45, 65-68. 1992.

[3] Naik, S.R., "Antioxidants and their role in biological function: An overview" Indian Drugs 40(9), 501-508. 2003.

[4] Zohary, D., and Hopf, M., "Domestication of plants in the old world (3rd ed.)" New York, NY: Oxford University Press. 2000.

[5] Ford, R., Redden, R.J., Materne, M.M., and Taylor, P.W.J., "Genome mapping and molecular breeding in lentil: Lentil. In: Kole, C. (Ed.), Genome Mapping and Molecular Breeding. Pulse, Sugar and Starch Crops" Heidelberg, Berlin, New York, Tokyo: Springer 3, 91-108. 2007.

[6] Pieniz, S., Colpo, E., Oliveira, V. R., Estefanel, V., and Andreazza, R., "Avaliação in vitro do potencial antioxidante de frutas e hortaliças" Ciência e Agrotecnologia 33(2), 552–559. 2009.

[7] Yan, X., Suzuki, M., Ohnishi-Kameyama, M., Sada, Y., Nakanishi, T., and Nagata, T. "Extraction and identification of antioxidants in the roots of yacon (Smallanthus sonchifolius)" J Agr Food Chem 47, 4711-4713. 1999.

[8] Zhang, B., Deng, Z., Ramdath, D. D., Tang, Y., Chen, P. X., Liu, R., Liu, Q., and Tsao, R., "Phenolic profiles of 20 Canadian lentil cultivars and their contribution to antioxidant activity and inhibitory effects on a-glucosidase and pancreatic lipase" Food Chem 172, 862-872. 2015.

[9] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C., "Antioxidant activity applying an improved ABTS radical cation decolorization assay" Free Radic Biol Med 26, 1231-1237. 1999.

[10] Benzie, I.F.F., and Strain, J.J., "The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': The FRAP assay" Anal Biochem 239, 70-76. 1996.

[11] Lee, S.J., Lee, S.Y., Ha, H.J., Cha, S.H., Lee, S.K., and Hur, S.J., "Rutin Attenuates lipopolysaccharide-induced nitric oxide production in macrophage cells" J Food Nutr Res 3(3), 202-205. 2015.

[12] Lee, S.J., Kim, Y.S., Hwang, J.W., Kim, E.K., Moon, S.H., Jeon, B.T., Jeon, Y.J., Kim, J.M., and Park, P.J., "Purification and characterization of a novel antioxidative peptide from duck skin by-products that protects liver against oxidative damage" Food Res Int 49, 285-295. 2012.

[13] Torino, M.I., Limón, R.I., Martínez-Villaluenga, C., Mäkinen, S., Pihlanto, A., Vidal-Valverde, C., and Frias J., "Antioxidant and antihypertensive properties of liquid and solid state fermented lentils" Food Chenm 136, 1030-1037. 2013.