Metabolomics Analysis of Cistus monspeliensis Leaf Extract on Energy Metabolism Activation in Human Intestinal Cells

doi: 10.1155/2012/428514

Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2012, Article ID 428514, 7 pages
doi:10.1155/2012/428514

Research Article

Metabolomics Analysis of *Cistus monspeliensis* Leaf Extract on Energy Metabolism Activation in Human Intestinal Cells

Yoichi Shimoda,[1] Junkyu Han,[1,2] Kiyokazu Kawada,[1,2] Abderrazak Smaoui,[3] and Hiroko Isoda[1,2]

[1] *Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan*
[2] *Alliance for Research on North Africa, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan*
[3] *Laboratory of Extremophile Plants, Center of Biotechnology of Borj-Cedria, 2050 Hammam-Lif, Tunisia*

Correspondence should be addressed to Hiroko Isoda, isoda.hiroko.ga@u.tsukuba.ac.jp

Received 1 September 2011; Revised 13 December 2011; Accepted 10 January 2012

Academic Editor: Munekazu Iinuma

Energy metabolism is a very important process to improve and maintain health from the point of view of physiology. It is well known that the intracellular ATP production is contributed to energy metabolism in cells. *Cistus monspeliensis* is widely used as tea, spices, and medical herb; however, it has not been focusing on the activation of energy metabolism. In this study, *C. monspeliensis* was investigated as the food resources by activation of energy metabolism in human intestinal epithelial cells. *C. monspeliensis* extract showed high antioxidant ability. In addition, the promotion of metabolites of glycolysis and TCA cycle was induced by *C. monspeliensis* treatment. These results suggest that *C. monspeliensis* extract has an ability to enhance the energy metabolism in human intestinal cells.

1. Introduction

A lot of natural herbs distributed around the Mediterranean Sea have been traditionally used by local people [1]. Most of the studies on plants to grow for Mediterranean climate have been investigated mainly in tolerance for the drying and relationship with environmental stress. However, the physiological function of natural herb is still poorly understood though many species grow allover the place. In an area of North Africa, the several species of natural herb were employed in traditional medicine as active against *Helicobacter pylori*, oxidative stress, hypertension, and hypoglycaemic [2–4]. *Cistus monspeliensis* is a perennial plant which in widely distributed from South Europe to North Africa. The genus *Cistus* is popular on tea and spice but not utilized for the antiaging effects. *C. monspeliensis* has been reported to have an antioxidation, antibacterial, and anti-inflammatory effects [5].

In the small intestine, goblet cells secrete mucus that forms a coating over the epithelial layer. The main function of epithelial layer includes absorption of food compounds [6]. The ingested capsaicin was carried into the intestinal epithelium, which is in contact with a high concentration of food ingredients [7, 8]. Considering the relevance of the food components and the effects of the oral route for human exposure, we have investigated the effect of capsaicin on the energy metabolism of intestine, by using Caco-2 cell line, a well-known *in vitro* model of intestinal epithelium [6].

The intracellular ATP accumulation is important for optimal integrity of the mucosa and has been suggested to play a specific role in the regulation of absorption and barrier functions [9, 10]. From these reports, it was supposed that the intracellular ATP accumulation contributes to the homeostasis of Caco-2 cells, because the regulation of absorption and barrier functions are necessary for Caco-2 cells differentiation [11].

In this study, the energy metabolism underlying the effect of *C. monspeliensis* extract on intestinal epithelium was clarified by performing the capillary electrophoresis time-of-flight mass spectrometry (CE-ToF/MS) analysis. Moreover, we performed real-time PCR to quantitate mRNA using the primers, related with ATP production, and the luciferase

assay was performed to measure intracellular ATP production in the intestinal epithelium. This is the first report that the extract of *C. monspeliensis* was induced by the activation of energy metabolism in human intestinal epithelial cells.

2. Materials and Methods

2.1. Cell Culture. Human intestinal epithelial Caco-2 cells were obtained from American Type Culture Collection (Manassas, VA, USA) and were used between passages 10 to 30. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO) supplemented with 10% (v/v) fetal bovine serum (Hyclone Co., Ltd.), 1% (v/v) penicillin-streptomycin (Lonza, Walkersville, MD USA), and 1% (v/v) nonessential amino acids (Cosmo Bio Co., Ltd., Tokyo, Japan) and incubated in an atmosphere of 5% CO_2 at 37°C.

2.2. Plant Materials and Extracts Preparation. 23 plants were collected between June and July in 2008 from Jendouba, Kairouan, and Kasserine areas in Tunisia and air-dried in the shade at room temperature and ground to powder with an electrical blender and stored at room temperature. Each plant sample (1 g) was extracted with 10 mL of distilled water at 105°C for 15 minutes or with 10 mL of 70% (w/v) ethanol at room temperature for 1 week. The extract water was filtered and sterilized using 0.22 μm membrane filter and stored at −80°C.

2.3. DPPH Assay. The antioxidant effect of plants was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay performed. DPPH (2 mg) was dissolved in ethanol (12.76 mL). The ethanol solution (6 mL), 400 mM 2-morpholinoethanesulfonic acid monohydrate (MES) solution (1.5 mL), and MilliQ water (4.5 mL) were mixed in one tube. Furthermore, plants extract (10 μL) and the mixing solution (190 μL) were added in 96-well plates and incubated for 10 min at room temperature. The absorbance was spectrophotometrically determined at 520 nm using a multidetection microplate reader (Powerscan HT, Dainippon Pharmaceutical, USA).

2.4. Total RNA Isolation and Real-Time PCR. After incubating seeded plates for 24 h, total RNA was purified using the ISOGEN kit (Nippon Gene Co., Ltd., Japan.) Total RNA was quantified by measurement at 260 nm with a UV spectrophotometer and was also measured at 280 nm to assess purity. Only RNA with a 260/280 ratio higher than 1.8 was used for the real-time PCR. Template cDNA was obtained from total RNA using the SuperScript reverse transcriptase system (Invitrogen). Briefly, RNA was denatured at 65°C for 5 min and incubated with 1 mL oligo (dT) 12–15 primers and chilled at 4°C. After adding SuperScript II reverse transcriptase (200 units), the reaction mix was incubated at 42°C for 60 min, then 10 min at 70°C. For the quantification of mRNA, nested primers were designed using Primer3Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Quantitative PCR reactions were performed in a MiniOpticon instrument (Bio-Rad, USA). Briefly, the RT mix (2 mL) was used as template for the real-time PCR mix containing 0.5 mM forward and reverse nested primers (2 μL each) and 2 × SYBR Green supermix (10 μL). The primers used were checked using the BLASTn program of the GeneBank; their sequence (TPI: forward: 5′-CTTGGCTGAGAGATGGAAGG-3′, reverse: 5′-CAGTGAAGGCAGACAAACCA-3′; PGM: forward: 5′-GCGGAGAACTTCATCCAGAG-3′, reverse: 5′-TGTCAGAATGATCCCACCAA-3′; ATP synthase: forward: 5′-CTGGAGGACCTGTTGATGCT-3′, reverse: 5′-TGGGGTTTTTCGATGACTTC-3′) was based on the known sequences in the cording region of the human genes. The amplification conditions were 3 min at 95°C, 10 sec at 95°C, 30 sec at 62°C, and 30 sec 72°C for 41 cycles. At the end of the reaction, a melting curve analysis was carried out to check for the presence of primer dimers.

2.5. Metabolomics Analysis. After incubating seeded plates for 24 h, the extract was added at 0.1% (w/v) concentration and the cells were incubated for 12 h. After treatment, 5% (w/w) mannitol solution was added at 10 mL and was removed. Once again, 5% (w/w) mannitol solution was added at 2.0 mL and was removed. Cells were scraped in 1.3 mL of methanol which includes 10 μM each of 2 internal standards (ISC1 and ISA1), and methanol including scraped cells was transferred at 1.0 mL to centrifuge tube. 1 mL of chloroform and 400 μL of MilliQ water were added to the solution and then thoroughly mixed. Followed by centrifugation at 2,300 g for 5 min at 4°C, the 250 μL of water layer was removed and centrifugally filtered through a 5-kDa-cutoff filter (Millipore, USA) to remove proteins. The filtrate was desiccated and dissolved in 20 μL of MilliQ water prior to injection. The capillary electrophoresis time-of-flight mass spectrometry (CE-ToF/MS) experiments were performed using an Agilent CE-ToF/MS system (Agilent Technologies Co.) unit 5. Separations were carried out on a fused silica capillary (50 μm i.d. × 80 cm total length). CE-ToF/MS conditions for anionic metabolites were followed. Run buffer: anion buffer solution (p/n: H3302-1021), rinse buffer: anion buffer solution (p/n: H3302-1022), sample injection: pressure injection 50 mbar, 25 sec, CE voltage: positive, 30 kV, MS ionization: ESI negative, MS capillary voltage: 3,500 V, MS scan range: *m/z* 50–1,000, sheath liquid (p/n: H3312-1020) [12].

2.6. ATP Measurement. ATP was assessed by firefly bioluminescence using the luminescence luciferase assay kit (TOYO Ink, Tokyo, Japan). Caco-2 cells were plated in 96-well plates at 1.0×10^6 cells/mL in 100 μL. After 24 h incubation, the extract was added at 0.1, 0.01, and 0.001% (w/v) concentration, and the cells were incubated for 3, 6, and 12 h. After treatment, 100 μL of luciferin-luciferase solution was added and stirred for 3 min using a microplate genie, 100 V (Scientific industries, Inc., USA). The luminescence was determined using a multidetection microplate reader (Powerscan HT, Dainippon Pharmaceutical, USA).

2.7. MTT Assay. The viability of cells was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide) assay. Briefly, Caco-2 cells were plated in 96-well plates at 1.0 × 106 cells/mL in 100 μL. After 24 h incubation, extract diluents with medium were added to obtain final concentrations from 0.1% to 0.001% (w/v), and the cells were cultured for 24 h, followed by the addition of 10 μL of 5.0 mg/mL of MTT. After 12 h incubation, 150 μL of 10% sodium dodecyl sulfate (Wako) was added and incubated for 48 h. The absorbance was spectrophotometrically determined at 570 nm using a multidetection microplate reader (Powerscan HT, Dainippon Pharmaceutical, USA).

3. Results

3.1. Effect of Antioxidant of 23 Plants Extracts. DPPH radical is well known as a stable organic free radical which has been used for estimation of the antioxidant capacity. DPPH radical is changed to nonradical to react antioxidants. We determined the antioxidation of 23 plants grow around the Mediterranean Sea. Especially, eighteen plants had higher antioxidation more than 75% (Table 1). We selected *C. monspeliensis* in twenty three plants that had high antioxidation to assess ATP production because this plant was used to traditional foods.

3.2. Effect of C. monspeliensis Extract on the Expression of TPI, PGM, and ATP Synthase mRNA by Caco-2 Cells. To investigate the effect of *C. monspeliensis* extract on the activation of energy metabolism in human intestinal epithelium (Figure 1), real-time PCR was employed to evaluate the mRNA expression of genes, related with glycolysis and TCA cycle. In this experiment, we used the primers of triosephosphate isomerase (TPI), phosphoglycerate mutase (PGM), and ATP synthase that have a deep relationship with intracellular ATP production and relatively higher expression in Caco-2 cells. Especially, to treat 0.1% (w/v) *C. monspeliensis* for 6 h, mRNA expression levels of TPI, PGM, and ATP synthase were upregulated by 170%, 161%, and 310%, respectively. PGM is an enzyme that catalyzes the internal transfer of a phosphate group from C-3 to C-2 which results in the conversion of 3-phosphoglycerate to 2-phosphoglycerate through a 2, 3-bisphosphoglycerate intermediate. TPI is an enzyme that catalyzes the reversible interconversion of the triose phosphate isomers, dihydroxyacetone phosphate, and D-glyceraldehyde 3-phosphate. TPI plays an important role in glycolysis and is essential for efficient energy production. ATP synthase is an important enzyme that creates energy for the cell to use through the synthesis of ATP in mitochondria. In Figure 1, mRNA expression of TPI and PGM, glycolytic enzymes, increased to treat with *C. monspeliensis* extract. Also, ATP synthase, electron transport chain enzyme, was increased.

3.3. Effect of C. monspeliensis Extract on the Metabolomics by Caco-2 Cells. As a result of the metabolomics analysis, *C. monspeliensis* extraction increased production of each metabolite in glycolysis and TCA cycle (Table 2). Glycolysis is a pathway of ATP production in anaerobic. In this result, three metabolites on rate-limiting step in glycolysis (glucose 6-phosphate, fructose 1,6-diphosphate, and pyruvic acid) were increased by *C. monspeliensis* extraction. Especially, pyruvic acid was the most increased in glycolytic metabolites. The production of pyruvic acid is related to promote function of ATPase that involved ATP synthesis [13]. ATP is not produced in TCA cycle; however, a lot of ATP is produced in electron transport chain. Antiaging on promoting energy metabolism is expected to promote function of glycolytic and electron transport chain enzymes that are pathways of ATP production.

Table 1: Effect of antioxidant of 23 plants extracts. The radical 2,2-diphenyl-1-picryhydrazyl (DPPH) was used for measuring the antioxidants. The hot water extract and the ethanol extract were diluted to 10 or 100 times by each solution. The values of antioxidant were calculated by the following formula: antioxidant (%) = $\{1-(\text{Absorbance}_{520}\ (\text{Sample})/\text{Absorbance}_{520}\ (\text{Control}))\}\times 100$.

Name of plants	EtOH extract	
	0.1% (w/v)	1% (w/v)
Ajuga iva	14	85
Artemisia campestris	38	91
Artemisia herba-alba	2	17
Cistus monspeliensis	24	93
Cyperus longus	10	50
Daphne gnidium	5	37
Erica multiflora	24	92
Globularia alypum	18	91
Laurus nobilis	10	54
Lavandula angustifolia	13	84
Lavandula officinalis	2	10
Lavandula stoechas	3	17
Marrubium vulgare	8	50
Mentha rotundifolia	8	43
Mentha viridis	12	71
Origanum majorana	13	85
Phillaria angustifolia	15	82
Pinus halepensis	11	53
Rhamnus lycioides	10	54
Teucrium polium	10	72
Thymus capitatus	13	87
Vitex agnus	13	78
Ziziphus lotus	15	76

3.4. Effect of C. monspeliensis on the Intracellular ATP Production by Caco-2 Cells. In the results of real-time PCR and metabolomics, *C. monspeliensis* was upregulated metabolite and mRNA expression involved ATP production. We determined ATP production of *C. monspeliensis* extract using the luminescence luciferase assay kit (Figure 2). 0.1, 0.01, and 0.001% (w/v) of *C. monspeliensis* extraction increased ATP production on Caco-2 compared with nontreated cells. Especially, to treat 0.01% (w/v) of *C. monspeliensis* for 3 h, ATP production was upregulated by 172%. As a result, we

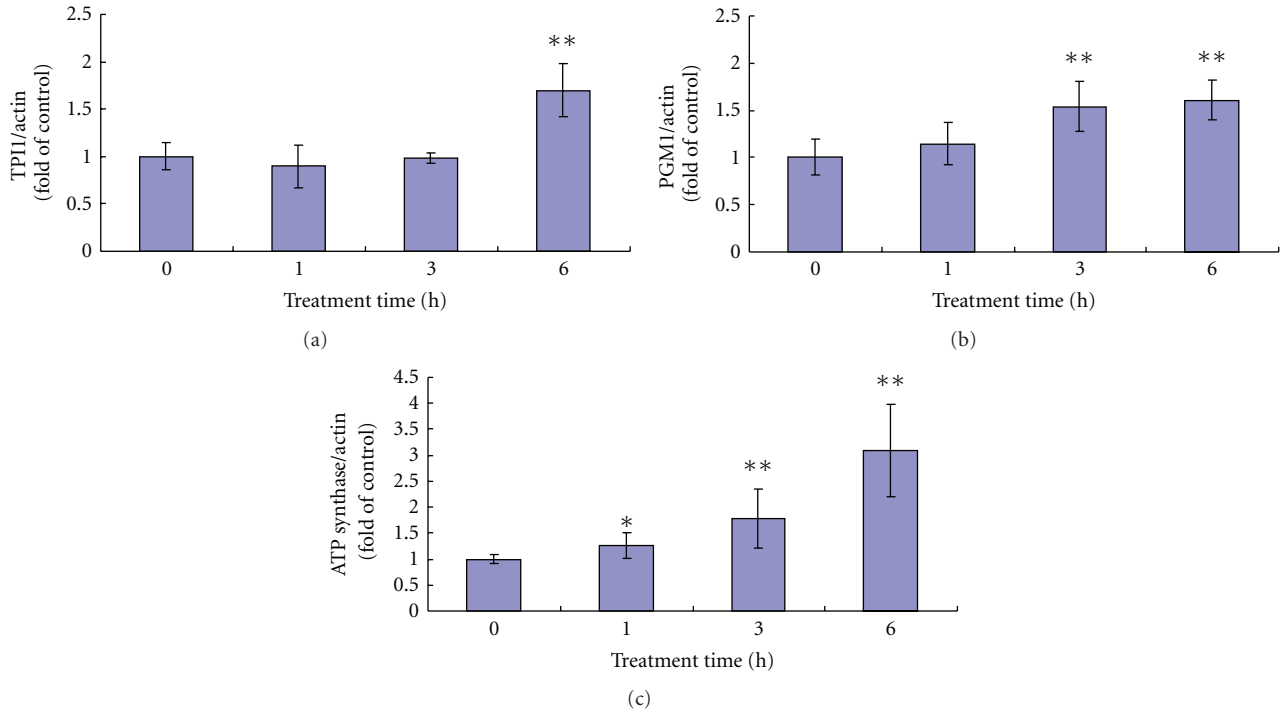

Figure 1: Effect of *C. monspeliensis* extract on the expression of TPI (a), PGM (b), and ATP synthase (c) mRNA by Caco-2 cells. β-actin was used as a house-keeping gene. The mRNA expression of TPI, PGM, and ATP synthase was normalized by β-actin mRNA expression. Each bar represents the mean ± SD ($n = 3$). $^*P < 0.05$, $^{**}P < 0.01$ compared to the control as determined by the t-test.

Table 2: Effect of *C. monspeliensis* extract on the metabolomics by Caco-2 cells. Quantified levels of metabolites involved in glycolysis pathway were determined. Caco-2 cells were treated with 0.1% (w/v) *C. monspeliensis* extract for 12 h. The ratio of quantified levels of metabolites was calculated by Caco-2 cells treated or nontreated with *C. monspeliensis* extract.

Metabolite	Ratio
Glycolysis	
Glucose 6-phosphate	1.7
Fructose 6-phosphate	1.6
Fructose 1,6-diphosphate	1.5
Dihydroxyacetone phosphate	2.1
3-Phosphoglyceric acid	1.0
Phosphoenolpyruvic acid	0.8
Pyruvic acid	4.2
TCA cycle	
Acetyl CoA_divalent	0.7
Citric acid	1.5
Cis-aconitic acid	1.4
Isocitric acid	1.0
2-Oxoglutaric acid	1.6
Succinic acid	0.8
Fumaric acid	1.1
Malic acid	1.0

considered that *C. monspeliensis* had effect of ATP production and to promote energy metabolism on Caco-2.

3.5. Effect of C. monspeliensis on the Cell Viability by Caco-2 Cells. We determined cell proliferation on Caco-2 using MTT assay. From the result of MTT assay, 0.01 and 0.001% (w/v) of *C. monspeliensis* extraction were not changed cell proliferation on Caco-2 cells, whereas 0.1% (w/v) of *C. monspeliensis* extraction induced the increase of cell proliferation on Caco-2 cells compared with nontreated cells. As a result, we considered that 0.1 to 0.001% (w/v) *C. monspeliensis* extract had not toxic effect for Caco-2 cells.

4. Discussion

ATP is a multifunctional nucleotide that is the most important as a "molecular currency" of intracellular energy transfer. In this role, ATP transports chemical energy within cells for metabolism. The glycolytic and TCA cyclic enzymes played an important role in the intracellular ATP production [14, 15]. TPI enzyme is essential for energy production, allowing two molecules of glyceraldehyde 3-phosphate to be produced for every glucose molecule, thereby doubling the energy yield. PGM enzyme catalyzes 1,3-bisphosphoglycerate and plays an important role downstream of glycolysis. The activity of TPI and PGM was not influenced by age or caloric restriction [16], while the other glycolytic enzymes were influenced. Furthermore, the activity and expression of these two enzymes, however, are

decreased by oxidation and disorders (Alzheimer's disease, hemolytic anemia, erythrocyte destruction, etc.) [17, 18]. These observations indicate that the expression and activity of these glycolytic enzymes were upregulated in response to specific signals, such as *C. monspeliensis* extraction. Furthermore, ATP synthase contains a rotary motor involved in biological energy conversion. ATP synthase uses the proton motive force to make ATP from ADP and inorganic phosphate (Pi) in mitochondria.

However, these enzymes have contribution toward not only ATP production but also regulation of cellular function. TPI isomerizes dihydroxy acetone phosphate (DHAP) to glyceraldehydes 3-phosphate (GAP). Its deficiency has been known to cause a severe multisystemic disease with autosomal recessive inheritance [19] and neurodegeneration [20]. Decreased TPI activity induces the accumulation of DHAP, which produces methylglyoxal instead of GAP. The methylglyoxal induces oxidative damage to proteins and DNA and accumulation of advanced glycation end products (AGEs), which leads to structural degeneration and functional decline of brain cells [21].

Also PGM is related with the apoptosis of human prostate cancer cells, LNCaP, DU145, and PC-3 [22]. Monoubiquitination of phosphoglycerate mutase, as well as formation of a noncovalent complex containing ubiquitin and phosphoglycerate mutase, increased in colorectal cancer, which may suggest a potential pathophysiological event [23]. A decreased level of phosphoglycerate mutase isoenzymes was reported in breast carcinoma [24] indicating its differential expression.

Active regulation of the mitochondrial ATP-synthase (complex V) in response to the cellular energy demand has been demonstrated in several species like rat, dog, and humans and different types of tissue like heart muscle, skeletal muscle, fibroblasts, and brain. Regulation of the ATP-synthase seems to be a central physiological phenomenon which is presumably present in many other species and other organs. As energy supply via the mitochondrial ATP-synthase plays such a vital role in almost every cell of the body, more diseases will probably be identified where (primary or secondary) abnormalities of this enzyme occur [25].

In addition, one of the causes of aging, free radical, is focused on oxidative cytotoxic. Especially, oxidative cytotoxic in mitochondrion is related to aging [26]. Mitochondrion that is important as metabolism organ of ATP production caused impairment accompanied effect of aging and ROS. Also, it is known that function of SOD decreased [27]. From this paper, the effect of enhancement component on ATP production can make use of antiaging effector as well as functional foods for antiaging.

Our research showed that *C. monspeliensis* extract induced the expression of enzymes, related with intracellular ATP production, in human intestinal epithelial cells. And *C. monspeliensis* extract enhanced the production of intracellular ATP. From these results, we suggest that *C. monspeliensis* extract can be used as antiaging effector. It is known that species of *Cistus* contain quercetin, kaempferol, aesculin, myricetin, and flavan-3-ols [28]. For example, quercetin has

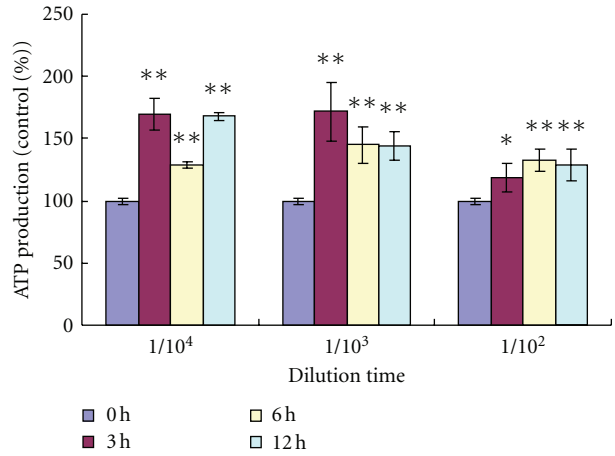

Figure 2: Effect of *C. monspeliensis* extract on the intracellular ATP production by Caco-2 cells. Caco-2 cells were treated with 0.1% to 0.001% (w/v) of *C. monspeliensis* extract for 3 h, 6 h, and 12 h. Each bar represents the mean ± SD ($n = 3$). $^*P < 0.05$, $^{**}P < 0.01$ compared to the control as determined by the *t*-test.

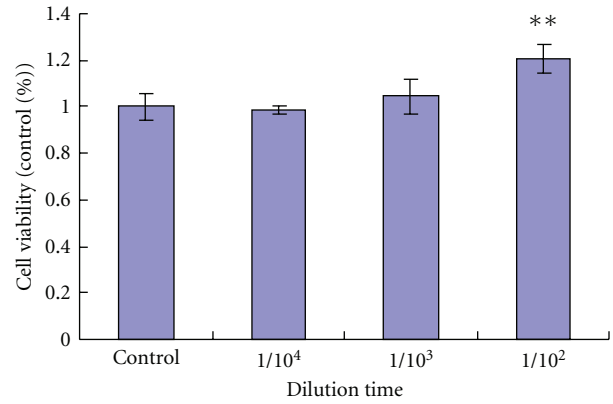

Figure 3: Effect of *C. monspeliensis* extract on the cell viability by Caco-2 cells. Caco-2 cells were treated with 0.1% to 0.001% (w/v) of *C. monspeliensis* extract for 24 h. Each bar represents the mean ± SD ($n = 3$). $^*P < 0.05$, $^{**}P < 0.01$ compared to the control as determined by the *t*-test.

antioxidation and has a function to improve memory impairment that accompany Alzheimer's disease [29]. Moreover, it is known that an aging is caused for decline of intestinal absorption [30, 31]. We expect that *C. monspeliensis* extract can contribute to prevent several diseases like senescence, Alzheimer's disease, cardiosclerosis, stenocardia, cancer, and so forth and recover the function of impairment intestinal absorption.

0.001, 0.01% (w/v) of *C. monspeliensis* increased ATP production (Figure 2) and, however, did not changed cell viability as compared with control (Figure 3). On the other hand, 0.1% (w/v) of *C. monspeliensis* decreased ATP production as compared with low-concentration sample and, however, up-regulated cell viability in MTT assay. From these results, we considered that ATP was consumed for cell proliferation in 0.1% (w/v) of *C. monspeliensis*, and

ATP accumulation a little decreased as compared with low concentration (0.01 and 0.001% (w/v)). It was reported that the ATP accumulation was contributed to the proliferation and homeostasis on Caco-2 cells [10]. We expect that *C. monspeliensis* extract can induce the ATP accumulation and then the activation of proliferation and homeostasis on Caco-2 cells.

We considered that isolation of active component from *C. monspeliensis* extract should be fulfilled and further *in vivo* studies should focus on the confirmation of activation and safety of *C. monspeliensis* extract.

5. Conclusion

Our findings indicate that *C. monspeliensis* extract had high antioxidant ability. In addition, we show that *C. monspeliensis* extract has the function of promoting energy metabolism pathways, including glycolysis, TCA cycle, and electron transport chain, in human intestinal epithelial cells. Also, *C. monspeliensis* extract enhanced the production of intracellular ATP on intestinal epithelium. From these results, *C. monspeliensis* extract has an ability to enhance the energy metabolism. Further studies are in progress to elucidate the effect of *C. monspeliensis* on antiaging.

Acknowledgment

This study partially was supported by the JICA-JST Science and Technology Research Partnership for Sustainable Development (SATREPS) Project: "Valorization of Bio-resources in Semi-Arid and Arid Land for Regional Development."

References

[1] M. E. González-Trujano, E. I. Peña, A. L. Martínez et al., "Evaluation of the antinociceptive effect of *Rosmarinus officinalis* L. using three different experimental models in rodents," *Journal of Ethnopharmacology*, vol. 111, no. 3, pp. 476–482, 2007.

[2] B. Kıvçak and S. Akay, "Quantitative determination of α-tocopherol in *Pistacia lentiscus*, *Pistacia lentiscus* var. *chia*, and *Pistacia terebinthus* by TLC-densitometry and colorimetry," *Fitoterapia*, vol. 76, no. 1, pp. 62–66, 2005.

[3] P. Barracosa, M. B. Lima, and A. Cravador, "Analysis of genetic diversity in Portuguese *Ceratonia siliqua* L. cultivars using RAPD and AFLP markers," *Scientia Horticulturae*, vol. 118, no. 3, pp. 189–199, 2008.

[4] I. C. F. R. Ferreira, L. Barros, M. E. Soares, M. L. Bastos, and J. A. Pereira, "Antioxidant activity and phenolic contents of *Olea europaea* L. leaves sprayed with different copper formulations," *Food Chemistry*, vol. 103, no. 1, pp. 188–195, 2007.

[5] H. Bouamama, T. Noël, J. Villard, A. Benharref, and M. Jana, "Antimicrobial activities of the leaf extracts of two Moroccan *Cistus* L. species," *Journal of Ethnopharmacology*, vol. 104, no. 1-2, pp. 104–107, 2006.

[6] Y. Sambuy, I. De Angelis, G. Ranaldi, M. L. Scarino, A. Stammati, and F. Zucco, "The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics," *Cell Biology and Toxicology*, vol. 21, no. 1, pp. 1–26, 2005.

[7] J. Szolcsányi, "Forty years in capsaicin research for sensory pharmacology and physiology," *Neuropeptides*, vol. 38, no. 6, pp. 377–384, 2004.

[8] M. Westerterp-Plantenga, K. Diepvens, A. M. C. P. Joosen, S. Bérubé-Parent, and A. Tremblay, "Metabolic effects of spices, teas, and caffeine," *Physiology and Behavior*, vol. 89, no. 1, pp. 85–91, 2006.

[9] H. Yang, J. Söderholm, J. Larsson et al., "Glutamine effects on permeability and ATP content of jejunal mucosa in starved rats," *Clinical Nutrition*, vol. 18, no. 5, pp. 301–306, 1999.

[10] L. J. Mandel, R. Bacallao, and G. Zampighi, "Uncoupling of the molecule "fence" and paracellular "gate" functions in epithelial tight junctions," *Nature*, vol. 361, no. 6412, pp. 552–555, 1993.

[11] J. Han and H. Isoda, "Capsaicin induced the upregulation of transcriptional and translational expression of glycolytic enzymes related to energy metabolism in human intestinal epithelial cells," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 23, pp. 11148–11153, 2009.

[12] T. Soga, Y. Ohashi, Y. Ueno, H. Naraoka, M. Tomita, and T. Nishioka, "Quantitative metabolome analysis using capillary electrophoresis mass spectrometry," *Journal of Proteome Research*, vol. 2, no. 5, pp. 488–494, 2003.

[13] A. Yokota, M. Henmi, N. Takaoka et al., "Enhancement of glucose metabolism in a pyruvic acid-hyperproducing *Escherichia coli* mutant defective in F1-ATPase activity," *Journal of Fermentation and Bioengineering*, vol. 83, no. 2, pp. 132–138, 1997.

[14] J. W. Kim and C. V. Dang, "Multifaceted roles of glycolytic enzymes," *Trends in Biochemical Sciences*, vol. 30, no. 3, pp. 142–150, 2005.

[15] T. Fleige, N. Pfaff, U. Gross, and W. Bohne, "Localisation of gluconeogenesis and tricarboxylic acid (TCA)-cycle enzymes and first functional analysis of the TCA cycle in *Toxoplasma gondii*," *International Journal for Parasitology*, vol. 38, no. 10, pp. 1121–1132, 2008.

[16] K. Hagopian, J. J. Ramsey, and R. Weindruch, "Influence of age and caloric restriction on liver glycolytic enzyme activities and metabolite concentrations in mice," *Experimental Gerontology*, vol. 38, no. 3, pp. 253–266, 2003.

[17] M. V. Martinov, A. G. Plotnikov, V. M. Vitvitsky, and F. I. Ataullakhanov, "Deficiencies of glycolytic enzymes as a possible cause of hemolytic anemia," *Biochimica et Biophysica Acta*, vol. 1474, no. 1, pp. 75–87, 2000.

[18] D. A. Butterfield, T. Reed, S. F. Newman, and R. Sultana, "Roles of amyloid β-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment," *Free Radical Biology and Medicine*, vol. 43, no. 5, pp. 658–677, 2007.

[19] C. Valentin, S. Pissard, J. Martin et al., "Triose phosphate isomerase deficiency in 3 French families: two novel null alleles, a frameshift mutation (TPI Alfortville) and an alteration in the initiation codon (TPI Paris)," *Blood*, vol. 96, no. 3, pp. 1130–1135, 2000.

[20] R. Bonnet, S. Pavlovic, J. Lehmann, and H. Rommelspacher, "The strong inhibition of triosephosphate isomerase by the natural β-carbolines may explain their neurotoxic actions," *Neuroscience*, vol. 127, no. 2, pp. 443–453, 2004.

[21] J. P. Gnerer, R. A. Kreber, and B. Ganetzky, "wasted away, a Drosophila mutation in triosephosphate isomerase, causes paralysis, neurodegeneration, and early death," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 41, pp. 14987–14993, 2006.

[22] N. K. Narayanan, B. A. Narayanan, and D. W. Nixon, "Resveratrol-induced cell growth inhibition and apoptosis is associated with modulation of phosphoglycerate mutase B in human prostate cancer cells: two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and mass spectrometry evaluation," *Cancer Detection and Prevention*, vol. 28, no. 6, pp. 443–452, 2004.

[23] L. W. Wattenberg, "Chemoprevention of cancer," *Cancer Research*, vol. 45, no. 1, pp. 1–8, 1985.

[24] N. Durany, J. Joseph, O. M. Jimenez et al., "Phosphoglycerate mutase, 2,3-bisphosphoglycerate phosphatase, creatine kinase and enolase activity and isoenzymes in breast carcinoma," *British Journal of Cancer*, vol. 82, no. 1, pp. 20–27, 2000.

[25] A. M. Das, "Regulation of the mitochondrial ATP-synthase in health and disease," *Molecular Genetics and Metabolism*, vol. 79, no. 2, pp. 71–82, 2003.

[26] E. Mariani, M. C. Polidori, A. Cherubini, and P. Mecocci, "Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview," *Journal of Chromatography B*, vol. 827, no. 1, pp. 65–75, 2005.

[27] Q. Meng, Y. T. Wong, J. Chen, and R. Ruan, "Age-related changes in mitochondrial function and antioxidative enzyme activity in fischer 344 rats," *Mechanisms of Ageing and Development*, vol. 128, no. 3, pp. 286–292, 2007.

[28] G. Attaguile, G. Perticone, G. Mania, F. Savoca, G. Pennisi, and S. Salomone, "*Cistus incanus* and *Cistus monspeliensis* inhibit the contractile response in isolated rat smooth muscle," *Journal of Ethnopharmacology*, vol. 92, no. 2-3, pp. 245–250, 2004.

[29] S. Tota, H. Awasthi, P. K. Kamat, C. Nath, and K. Hanif, "Protective effect of quercetin against intracerebral streptozotocin induced reduction in cerebral blood flow and impairment of memory in mice," *Behavioural Brain Research*, vol. 209, no. 1, pp. 73–79, 2010.

[30] T. Woudstra and A. B. R. Thomson, "Nutrient absorption and intestinal adaptation with ageing," *Best Practice and Research in Clinical Gastroenterology*, vol. 16, no. 1, pp. 1–15, 2002.

[31] C. G. MacIntosh, M. Horowitz, M. A. M. T. Verhagen et al., "Effect of small intestinal nutrient infusion on appetite, gastrointestinal hormone release, and gastric myoelectrical activity in young and older men," *American Journal of Gastroenterology*, vol. 96, no. 4, pp. 997–1007, 2001.