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Rebuttal on Comparison of Protective Effects between Cultured *Cordyceps militaris* and Natural *Cordyceps sinensis* against Oxidative Damage

We very much appreciate the valuable comments by Dr. Hamburger on our previous publication, "Comparison of Protective Effects between Cultured *Cordyceps militaris* and Natural *Cordyceps sinensis* against Oxidative Damage" (*1*). We have carefully reviewed our paper and have the following responses.

First, Dr. Hamburger points out that our work lacks any information on the origin and the taxonomic characterization of the fungal strains. On this basis, Dr. Hamburger also points out the term "cultured *Cordyceps sinensis*" is misleading and should be replaced by a correct taxonomic assignment. As described under Materials and Methods in our original publication (*1*), *Cordyceps sinensis* and *Cordyceps militaris* were kindly donated by the Iso-Green Biotechnology Co. Ltd., Danshui Town, Taipei County, Taiwan. For certain commercial reasons, this company refused to provide the molecular phylogenetic data of the fungal strains for us. Furthermore, the designations "cultured *Cordyceps militaris*" and "natural *Cordyceps sinensis*" and not "cultured *Cordyceps sinensis*" were used in our study.

Second, Dr. Hamburger points out a problem about the inadequate characterization of the chemical composition of the examined samples. He also notes that compound classes, which are critical for the paper, such as the phenolic constituents, are not analyzed by HPLC. Li and others in 2006 showed nucleosides, polysaccharide, ergosterol, and mannitol are significant markers for quality control of *Cordyceps* (*2*). Among these ingredients, cordycepin and adenosine are the major nucleosides in *Cordyceps*. In our paper (*1*), Table 1 showed the contents of cordycepin and adenosine in water extracts of *C. militaris* and *C. sinensis*, respectively. As can be seen in Figure 4, the other main chemical ingredients such as uridine in *Cordyceps* were analyzed by HPLC. Except for these major ingredients, we agree that other unknown bioactive compounds present in *C. militaris* and *C. sinensis*, respectively, contribute to *Cordyceps'* biological activities. To compare the antioxidant efficiency of water extracts of *C. militaris* and *C. sinensis* is still the major goal of our study. We did not characterize the explicit chemical composition of the examined samples. Furthermore, the levels of polyphenolics in water extracts of *C. militaris* and *C. sinensis* were relatively low compared to those in green tea, pu-erh tea, and roasted coffee residue in our previous studies (*3*, *4*). These data implied phenolic constituents would not be the major active ingredients present in samples. Therefore, the dominant polyphenolics were not further analyzed by HPLC.

Third, Dr. Hamburger points out that an obsolete and nonspecific colorimetric test was used for quantitative determination of radical scavenging phenolics. Dr. Hamburger also states that a possible, and much more reasonable, explanation for the outcome of the colorimetric test is the occurrence of pyridone alkaloids and tetramic acids in the extracts. Although we largely agree with his points, the TEAC method was extensively used to detect the antioxidant capacity of food products and drugs. There are many laboratories still using the TEAC method to screen the total antioxidant capacity of compounds. Also, in our study, the tested samples were prepared from water-soluble constituents of *C. militaris* and *C. sinensis*, respectively. Schmidt and others in 2003 isolated and identified tetramic acids and pyridone alkaloids from a methanol-soluble, not water-soluble, portion of *Paecilomyces militaris* (*5*). Because these are different extracting processes, we assume tetramic acids and pyridone alkaloids could not exist in water extracts of *C. militaris* and *C. sinensis* and provide a positive color reaction of "flavonoids" in the colorimetric test. On the other hand, in the study in question (*1*), we tried to determine whether flavonoids are present in *C. militaris* and *C. sinensis* by the method set up by other researchers. According to the data gained, the levels of flavonoid in water extracts of *C. militaris* (CME) and *C. sinensis* (CSE) are relatively low compared to other resources (*3*, *4*). This implied a few flavonoids were present in samples CSE and CME, respectively. We also agree with the point by Dr. Hamburger that flavonoids have been generally considered to be a compound class that occurs only in higher plants. However, evidence for the presence of flavonoids in CME and CSE is lacking; the identification of flavonoids present in CME and CSE needs to be further researched. According to the data gained from our model system, we could not exclude the possibility of a few flavonoids present in CSE and CME, respectively.

Dr. Hamburger commented on an overinterpretation of biological or pharmacological data in our paper. He also commented that one could not extrapolate to a possible clinical benefit based on preliminary laboratory data. Our paper was only a preliminary laboratory study and offers some scientific data to researchers who are interested. Although the title of our manuscript is imprecise, the content of the paper clearly describes the results of the study and would not confuse readers. In addition, the mechanisms of various aging diseases are related to superabundant and harmful oxidative stress production in the living body. Many studies have suggested that antioxidants can possibly prevent and delay the progression of aging diseases. Our preliminary laboratory data in this paper might provide some opinions for the in vivo experiment.

LITERATURE CITED

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