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Antrodia Camphorata Declines Oxidative Stress and Enhances Antioxidant Enzyme Activity in the Brain Cortex of Rats

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Abstract: Traditional Chinese Medicine of Antrodia camphorata has been commonly used as complementary and alternative medicines in Taiwan and Asia countries due to a variety of beneficial effects such as anti-inflammatory and anti-oxidation. Biological effects of Antrodia camphorata on oxidative stress and antioxidant enzyme activity in the brain cortex, however, have not yet fully defined so far. Experimentally, twenty male Sprague-Dawley rats were randomly divided into control and experimental subject. Control and experimental rats were intraperitoneally injected with normal saline and Antrodia camphorata for consecutive 14 days, respectively. On day 15, the brain cortex was harvested and homogenates for further biochemical analysis of lipid peroxidation and antioxidant enzyme activity. Our present data showed that Antrodia camphorata can significantly ($P < 0.05$) reduce the magnitude of oxidative stress as presented by a declined malondialdehyde (MDA) concentration in the present work. In addition, a remarkable ($P < 0.05$) enhancement of the antioxidant enzyme activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) was found in Antrodia camphorate-treated rats as compared to control subject. In conclusion, our experimental finding demonstrates the fact that neuroprotective effects of Antrodia camphorata are correlated with reducing oxidative stress and enhancing antioxidant enzyme activity in the brain cortex of rats.

Keywords: Antrodia Camphorata, Brain Cortex, Oxidative Stress, Antioxidant Enzyme Activity

1. Introduction

Traditional Chinese Medicine of Antrodia camphorata has been commonly used as the complementary and alternative medicines in Taiwan [1]. Antrodia camphorata has long been used for treating diarrhea, hypertension, abdominal pain, chemical intoxication, and especially for treatment of liver disease in Taiwan [2-4]. Previous study has indicated that Antrodia camphorata can significantly induce apoptosis on human promyelocytic leukemia (HL-60) cells [5]. In addition, study has pointed out that Antrodia camphorata possesses free radical scavenging ability in reducing chemical-induced hepatic lesion [6]. Moreover, Antrodia camphorata can decline hydrogen peroxide induced oxidative damage and enhance hepatic glutathione-dependent enzymes upon protecting CCl4-induced damage on rat liver [7]. Additional investigation has evidenced that Antrodia camphorata possesses neuroprotective effect against cerebral ischemia in the in vivo experiment by means of reducing infarct volume and improves neurobehavioral scores [8-12].

Lipid peroxidation is known a complex reactive oxygen species (ROS) inducing reaction caused by the degradation of the component of polyunsaturated fatty acid (PUFA) in cells [13-15]. Moreover, intensity of oxidative stress is in paralleled with the malondialdehyde (MDA) level has also been well-realized [16-17]. In this regard, higher MDA level represents elevated oxidative stress and injury has been well accepted. It has been well recognized that equilibrium between the status of oxidant and antioxidant is crucial for living organisms in

alleviating oxidative attack [18]. It is also recognized that the brain tissue is extremely sensitive to the oxidative attack [18-19]. Therefore, proper antioxidant activity is necessary for the brain tissue in attenuating further oxidative lesion. There are three mainly antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) mainly existed in the brain tissue for alleviating oxidative attack [18-19]. To date, the biological effects of Antrodia camphorata on oxidative stress and antioxidant enzyme activity of SOD, GPX, and CAT in the brain cortex, however, have not been fully investigated and needs to be further elucidated in this present experiment.

2. Materials and Methods

2.1. Experimental Animal Treatment

In this present study, twenty male Sprague-Dawley rats, weighing from 220-270 g were encompassed in this work. All animals were purchased from National Laboratory Animal Breeding and Research Center (Taiwan). Experimental rats were kept in stainless-steel mesh cages, housed under controlled conditions (22±2°C, 50±20% relative humidity, 12-h light-dark cycle) with diet and water. All animal handling and experimental protocols were approved by Institutional Animal Care and Use Committee (IACUC) of Central Taiwan University of Science and Technology. Experimentally, rats were randomly divided into control and experimental group of 10 rats each. Rats were intraperitoneally injected with 0.5ml/kg of normal saline and Antrodia camphorata once in a day for consecutive fourteen days, respectively. On day fifteen, the brain cortex was isolated and homogenates for further biochemical analysis.

2.2. Determination of the Malondialdehyde (MDA) Level in the Homogenates of the Brain Cortex

Right brain cortex (0.5 g, wet weight) was removed and then homogenized in 5 ml of cold KCl (154 mM) solution by motor driven tissue homogenizers with Teflon pestles and followed by centrifuged at 4°C for 15 min at 10000 g. The supernatants were collected for the measurement of the MDA level. The reagent of 1,1,3,3-tetraethoxypropane (TEP) was used as a standard solution in the reaction with thiobarbituric acid reactive substance (TBARS). Reagent of thiobarbituric acid (TBA) was obtained from E. Merck (Germany) and used for MDA analysis. Reagent of 1,1,3,3-tetraethoxypropane (TEP) was used as a standard solution in the reaction with thiobarbituric acid reactive substance (TBARS). Basically, the analytical principle of this method based on the determination of the pink color that is produced by the interaction of TBA with the component of MDA. A wavelength of 532 nm was selected for analyzing the MDA level using spectrophotometer (U-1900, Hitachi, Japan).

2.3. Antioxidant Enzyme Activity of CAT, SOD, and GPX in the Homogenates of the Brain Cortex

Right brain cortex was homogenized in 5 ml of cold KCl (154 mM) solution by motor driven tissue homogenizers with Teflon pestles and followed by centrifuged at 4°C for 15 min at 10000 g. The supernatants were harvested for the analysis of the enzyme activity of CAT, SOD, and GPX. Basically, the CAT activity was measured according to the Cayman catalase assay kit (Cayman Chemical Company, USA). The analytical principle is based on the reaction of the enzyme with methanol in the presence of hydrogen peroxide. The chromogen of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole was measured with the produced Formaldehyde. The SOD activity was determined according to the method of Cayman superoxide dismutase assay kit (Cayman Chemical Company, USA). Detective principle of the GPX activity is that the GPX can catalyze the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted into reduced form with the concomitant oxidation of NADPH to $NADP^+$, and the decrease in absorbance at the wavelength of 340 nm was measured. Antioxidant activities of CAT, SOD, and GPX were determined by means of spectrophotometer (Thermo Scientific Multiskan Spectrum, USA).

2.4. Statistical Analysis

All experimental results were expressed as mean ± SD. They were statistically analyzed by using Mann–Whitney U Test. Statistical differences were considered significant at a p value less than 0.05 in this present experiment.

3. Results

3.1. Malondialdehyde (MDA) Level in the Homogenates of the Brain Cortex

It has also been considered that the intensity of lipid peroxidation is positively correlated with the malondialdehyde (MDA) level. Given the fact, higher MDA level represents higher lipid peroxidation and oxidative stress. Our present observation indicated that the MDA level in the control and the Antrodia camphorata-treated subject was 16 ± 4 and 12 ± 2.5 μM, respectively. Compared with the control group, a significant reduction ($P < 0.05$) of the MDA level was observed in Antrodia camphorata administrated rats as shown in Figure 1.

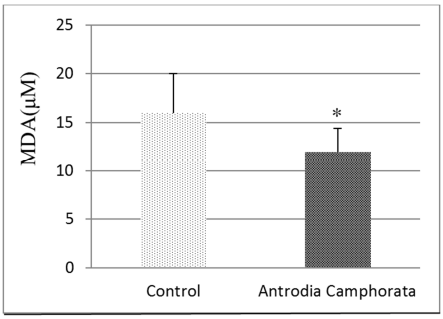

Fig. 1. *Profiles of the MDA level in the brain cortex of rats. Data were expressed as mean ± S.D. (N=10). They were statistically analyzed by using Mann–Whitney U Test. Statistical differences were considered significant at a p value less than 0.05.*

3.2. Antioxidant Enzyme Activity of SOD in the Homogenates of the Brain Cortex

Experimentally, our present data showed that antioxidant enzyme activity of SOD in the control and the Antrodia camphorata subject was 0.7 ± 0.1 and 0.8 ± 0.05 U/ml, respectively. Compared with the control group, a significant increase ($P < 0.05$) of the SOD activity was found in Antrodia camphorata-treated rats as listed in Figure 2.

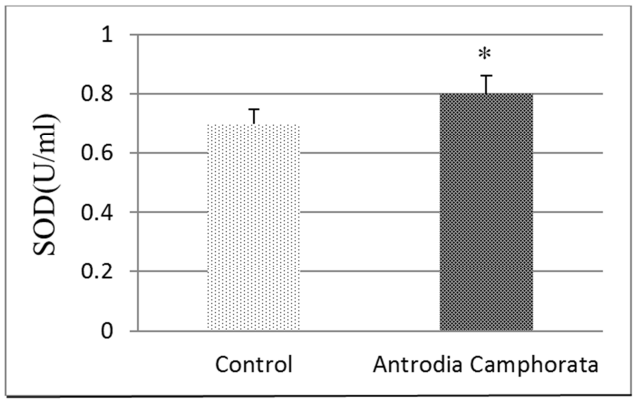

Fig. 2. *Profiles of the SOD activity in the brain cortex of rats. Data were expressed as mean ± S.D. (N=10). Statistically method of Mann–Whitney U Test was used. Statistical differences were considered significant at a p value less than 0.05.*

3.3. Antioxidant Enzyme Activity of CAT in the Homogenates of the Brain Cortex

For the analysis of CAT activity, level of control and Antrodia camphorata-treated group was 31.7 ± 4.7 and 38.4 ± 5.0 nmol/ml, respectively. A significant ($P < 0.05$) enhancement of the CAT activity was seen in Antrodia camphorata treated group as compared to the control subject as showed in Figure 3.

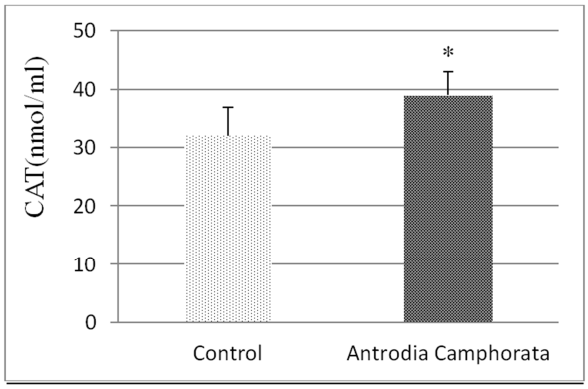

Fig. 3. *Profiles of CAT activity in the brain cortex of rats. Data were expressed as mean ± S.D. (N=10). Statistical method of Mann–Whitney U Test was used in the present study. Statistical differences were considered significant at a p value less than 0.05.*

3.4. Antioxidant Enzyme Activity of GPX in the Homogenates of the Brain Cortex

Similarly, the GPX activity in the control and the Antrodia camphorata subject was 107 ± 10 and 122 ± 16 nmol/mim/ml, respectively. An obvious ($P < 0.05$) elevation of the GPX activity in Antrodia camphorate-treated subject was observed as compared to the control group as presented in Figure 4.

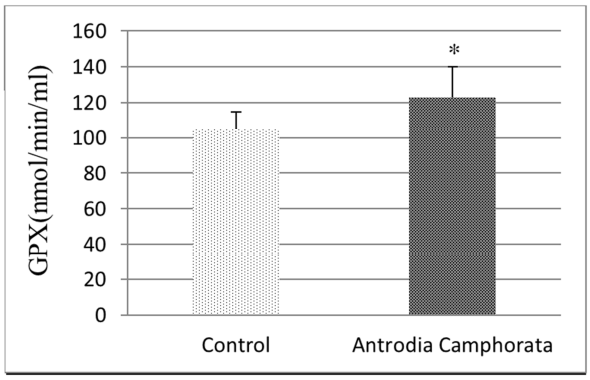

Fig. 4. *Profiles of GPX activity in the brain cortex of rats. Data were expressed as mean ± S.D. (N=10). Statistical method of this study was the Mann–Whitney U Test. Statistical differences were considered significant at a p value less than 0.05.*

4. Discussion

The Chinese Medicine of Antrodia camphorata has been reported in possessing extensive biological activities such as hepatoprotection, immune modulation, anti-inflammation, anticancer, free radicals scavenger, and antioxidant activity [1-3]. In vivo study has revealed that Antrodia camphorata shows antioxidant effects against H_2O_2-induced cytotoxicity in HepG2 in carbon tetrachloride- (CCl4-) induced hepatotoxicity [7]. In addition, Antrodia camphorata may play an important role in preventing oxidative lesion by up-regulating the hepatic glutathione-dependent enzymes in living systems to preserve the normal reduced and oxidized glutathione (GSH/GSSH) ratio [5]. Investigation has reported that mycelia of Antrodia camphorata could exhibit anti-hepatitis B virus effect [8]. Furthermore, Antrodia camphorata could inhibit N-formyl-methionylleucyl-phenylalanine (fMLP) or phorbol 12-myristate 13- acetate- (PMA-) induced ROS production in peripheral human neutrophils has been reported previously [6]. Our present study clearly showed that Antrodia camphorata can significantly reduce lipid peroxidation level in the brain cortex of rats (Figure 1). In fact, lipid peroxidation processes are also known a complex ROS inducing reaction caused by the degradation of polyunsaturated fatty acid in cells. In this regard, higher MDA level represents elevated oxidative stress and injury has been well-accepted. Our present observation proposed the possibility here that the beneficial effect of Antrodia camphorata may be involved in the inhibition of free radical formation, and our present finding was in according with the previous investigation [3].

It has been well known that the aerobic organisms, which derive their energy from the reduction of oxygen, are susceptible to reactive oxygen species such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals that inevitably form during the metabolism of oxygen. There are a

variety of diseases have been reported in correlated with the damage of reactive oxygen species [2-4]. Indeed, once an imbalance between higher free radical-generating and lower radical-scavenging systems was observed, the oxidative stress occurs. As mentioned above, it is important to note that dynamically equilibrium between the status of oxidant and antioxidant is important for living organisms in alleviating oxidative attack. Brain tissue is well recognized to be extremely sensitive to oxidative attack. In this regard, proper antioxidant activity is thinkable to be important to the brain. Antioxidant enzymes of superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) are crucial for protecting brain tissue from reactive oxygen species attack [14, 18]. Antioxidant enzyme of SOD catalyzes superoxide radicals to oxygen and hydrogen peroxide (H_2O_2) [14]. For CAT enzyme, it converts H_2O_2 into water and oxygen. The GPX converts H_2O_2 into non-toxic substances of water and oxygen. Based on high levels of produced reactive oxygen species result from high aerobic metabolism, the brain tissue is susceptible to oxidative lesion [13]. Therefore, higher generated reactive oxygen species may react with the brain tissue and resulting oxidative damage to the brain. Under normal situations, appropriate antioxidant enzyme activity is important for the brain in preventing further oxidative damage mediated by toxic free radicals. Our present observation demonstrated that the Chinese Medicine of Antrodia camphorata can significantly enhance antioxidant enzyme activity of SOD in the brain cortex as compared to the control subject. Previous in vitro study has also revealed that Antrodia camphorata can enhance SOD activity [2]. In addition, previous study has revealed the fact that decreased SOD activity was observed in cerebral ischemic brain, and our present finding was in agreement with the former investigation [20]. Antioxidant enzyme of CAT is responsible for the detoxification by converting hydrogen peroxide into water and oxygen within the brain tissue. Hence, increased CAT activity is helpful for the brain in attenuating oxidative injury and on the contrary, reduced CAT activity is reported to be exacerbating the brain functions [20]. Figure 3 showed that the CAT activity in the homogenates of the brain cortex in Antrodia camphorata-treated rats was statistically elevated as compared with the control subject. Previous research has suggested that elevated CAT activity is beneficial for the brain in declining further oxidative lesion and our present finding was in agreement with the previous observation [20]. It has been well-recognized that biological function of the GPX enzyme can convert the toxic molecule of hydrogen peroxide (H_2O_2) into water and oxygen. Previous study has pointed out that increased GPX activity can prevent the brain tissue from further oxidative lesion [3, 5]. In this in vivo experiment, an increased GPX activity was found in Antrodia camphorata treated rats, and our present finding was in accordance with the former investigations [13, 14]. In addition, recent in vivo study has indicated that Antrodia camphorata potentiates neuroprotective effects [21]. Accordingly, it is of note that Chinese Medicine of Antrodia camphorata seems to possess multiple biological properties in protecting brain tissues from further oxidative attack.

5. Conclusion

Biological efficacies of Antrodia camphorata on oxidative stress and antioxidant enzyme activity in the brain, however, have not yet fully defined to date. Taking all evidence together, our experimental findings manifest the fact that Chinese Medicine of Antrodia camphorata may exert its neuroprotective effect by means of reducing oxidative stress as presented by a decreased lipid peroxidation level and elevating antioxidant enzyme activity in the brain cortex. Importantly, it seems possible to speculate that Chinese Medicine of Antrodia camphorata may be can considered a medicinal potential in the near future in preventing brain tissues from further oxidative attack such as cerebral ischemic insult.

Acknowledgements

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