Effect of parboiled germinated brown rice on rat brain hippocampus lipid peroxidation and superoxide dismutase activity in an animal model of oxidative stress

Kansuda Wunjuntuk[1*], Jureeporn Nounmusig[2] and Aikkarach Kettawan[3]

ABSTRACT

Parboiled germinated brown rice (PGBR) from Khao Dawk Mali 105 (KDML 105) Thai rice variety is well-known for its nutrients and bioactive compounds. This study was conducted to determine the neuroprotective effect of PGBR on carbon tetrachloride (CCl_4)-induced brain oxidative stress. Thirty-five male Sprague-Dawley rats were randomly divided into five groups: (1) control group (basal diet), (2) CCl_4 group (basal diet+CCl_4), (3) white rice (WR) group (WR diet+CCl_4), (4) brown rice (BR) group (BR diet+CCl_4), and (5) PGBR group (PGBR diet+CCl_4). The corn starch in AIN76A basal diet was replaced by cooked PGBR, BR and WR powders for preparing PGBR, BR and WR diets, respectively. Each rat in group 2, 3, 4 and 5 was orally administered with 1 mL/kg rat of CCl_4 in olive oil (1:1, v/v) twice a week. After 8 weeks, all rats were sacrificed under CO_2 anesthesia. The hippocampus was dissected and homogenized to evaluate the level of lipid peroxidation product (malondialdehyde, MDA) and antioxidant enzyme activity (superoxide dismutase, SOD). CCl_4-treated rats had significantly higher brain hippocampus MDA level and significantly lower SOD activity than the control group. However, rats in PGBR group showed the neuroprotective effects as indicated by a decrease in brain hippocampus MDA level and an increase in SOD activity in comparison with the CCl_4 group. This finding suggests that PGBR may prevent CCl_4-induced brain hippocampus oxidative stress due to its antioxidant activity and bioactive compounds.

Keyword: parboiled germinated brown rice, brain, lipid peroxidation, superoxide dismutase, carbon tetrachloride

[*]Corresponding author; Tel.: +66 2 579 5534 Ext.104; e-mail address: fagrkdw@ku.ac.th, noinadekdee@gmail.com

[1]Department of Home Economics, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand

[2]Public health program, Faculty of science and technology, Nakhon Si Thammarat Rajabhat University, 80280, Thailand

[3]Food Chemistry Division, Institute of Nutrition, Mahidol University, Nakhon Pathom, 73170, Thailand

INTRODUCTION

Brown rice or paddy rice is soaked for water absorption and followed with germination under certain conditions till the sprout length is 0.5 – 1.0 mm. Several of valuable nutrients was remarkably increased after germination, especially increasing 13-fold for γ-oryzanol, 10-fold for γ-aminobutyric acid (GABA), about 4-fold for dietary fiber, vitamin E, niacin and lysine, and nearly 3-fold for vitamins B1 and B6, and magnesium (Kayahara *et al*., 2000). Furthermore, previous studies have shown the anti-oxidant, anti-inflammation, anti-diabetic and anti-cancer properties in germinated brown rice (Latifah *et al*., 2010; Zhang *et al*., 2010; Imam *et al*., 2012; Soiampornkul *et al*., 2012). Parboiled germinated brown rice (PGBR) was produced from Khao Dawk Mali 105 (KDML 105) rice variety, which is the most famous Thai rice, and is known for its texture and aroma. PGBR was produced by steaming germinated paddy rice. During parboiling, starch granules of rice are gelatinized, which improves stability and lessens the rupture of rice grain. Our recent reports (Wunjuntuk *et al*., 2016a; Wunjuntuk *et al*., 2016b) showed the high quantity of GABA, γ-tocotrienol, γ-oryzanol and ferulic acid in PGBR of Khao Dawk Mali 105 rice variety, KDML 105 *(O. sativa* L. cv.) grown in Thailand. In addition, our results showed that PGBR had more anti-fibrotic, anti-inflammatory and antioxidant capacities in rat's liver than brown rice and white rice.

It was evident that oxidative stress played a crucial role in neurodegeneration, which is an important player in the pathophysiology of brain diseases such as Alzheimer's diseases, Parkinson's disease, stroke and multiple sclerosis (Popa-Wagner *et al*., 2013). The brain consumes 20% of the body's oxygen for aerobic metabolism leading to the augment of metabolic rate and a large amount of ROS. Neuronal cells are rich in polyunsaturated fatty acid (PUFA), which is the major target of ROS involving hydrogen abstraction from a carbon-carbon double bonds. With further oxygen addition, lipid peroxyl radicals and hydroperoxides (primary oxidation products) are generated. Subsequently, the secondary oxidation products including malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE) are created (Ayala *et al*., 2014). SOD, an important protective enzyme of the brain, plays a prominent role in the inactivation of superoxide radicals that could inhibit the initiation of lipid peroxidation by ROS. The elevation of MDA and the depression of SOD activity may reflect the brain injury (Popa-Wagner *et al*., 2013). Carbon tetrachloride (CCl_4) is a well-known model for detecting hepatoprotective properties of natural components. In addition, CCl_4 causes free radical generation in many tissues such as liver, kidney, heart, lung, testis, blood and brain (Dashti *et al*.,1989). Therefore, the main aim of present work was to evaluate the effect of parboiled germinated brown rice on rat brain hippocampus lipid peroxidation and SOD activity in CCl_4-induced oxidative stress model.

MATERIALS AND METHODS

1. Animals

This animal study was approved by the Experimental animal care and use committee, The National Laboratory Animal Centre, Mahidol University (approval no. RA2013-04). A total of 35 Male Sprague–Dawley rats weighing 200 –250 g was obtained from The National Laboratory Animal Centre, Mahidol University and maintained in an environmentally controlled room (23±2°C, 50±20% humidity) with a 12-hour light/dark cycle.

2. Preparation of parboiled germinated brown rice

PGBR, BR and WR were produced from the same kind of Khao Dawk Mali 105 (*Oryza sativa* L. cv., KDML 105) rice grown in Thailand. PGBR was prepared according to the method of Wunjuntuk *et al*. (2016b) using steamed germinated rice before milling. In brief, the paddy rice was soaked in water for 18 h. The soaked rice was removed from an automatic pre-germination machine (Thai Patent pending No. 1001000300) and then germinated inside the chamber for about 2 days. For the parboiling process, the germinated rice was steamed for 30 min and dehydrated by vacuum drying at 70–75 °C for 2 h. Finally, the steamed rice was dried in hot air oven at 40 °C until the moisture content reached to 13%.

3. Preparation of animal diets

All rice was cooked in an electric rice cooker (1.8 L, Sharp KS-19ET) using the appropriate method (Wunjuntuk *et al*., 2016a), freeze dried, ground into fine powder, packed in vacuum-aluminum foil bags and stored in -20 °C until used. The corn starch of AIN76A (basal diet) was replaced with freeze dried cooked white rice for WR diet, or replaced with freeze dried cooked brown rice for BR diet, or replaced with freeze dried cooked parboiled germinated brown rice for PGBR diet. The contents of WR, BR, and PGBR added into the diet were based on the composition of energy, protein, carbohydrate, and fat.

4. Experimental design

After the acclimation, rats were randomly divided into five experimental groups, each containing 7 rats. Group I rats were fed with the basal diet (AIN-76A), which was referred as the control group. Group II rats were fed with the basal diet, and orally administrated with 1 ml of CCl_4/olive oil mixture (1:1, v/v)/kg body weight twice a week for consecutive 8 weeks. Group III, IV and V rats were fed with PGBR, BR and WR diets, and orally administrated with 1 ml of CCl_4/olive oil mixture (1:1, v/v)/kg body weight twice a week for consecutive 8 weeks. All rats were allowed to access freely to the experimental diets and water. At the end of 8 weeks, all rats were fasted overnight and sacrificed under CO_2 anesthesia. The brain samples were immediately dipped into liquid nitrogen and stored at -80 °C until analysis.

5. Brain homogenate preparation

The brain hippocampus was homogenized by manual homogenizer with ice-cold Tris-HCl buffer (100 mM, pH 7.4) containing 5 mM EDTA and 1% (v/v) Triton X-100 to give a final concentration of 10% w/v and sonicated on ice for 10 min. The homogenate solution was centrifuged at 3,000x g for 10 min at 4°C (ROTINA 38, Tuttlingen, Germany). The supernatant was collected and stored at -80°C. The protein content assay was determined by bicinochoninic acid (BCA) protein assay (Smith *et al*., 1985) using BSA (bovine serum albumin) (Bio-Rad Laboratories, CA, USA) as a standard and using Synergy^{TM} HT microplate reader (BioTek, Vermont, USA) for detection.

6. Measurement of MDA level

Lipid peroxidation of homogenized brain hippocampus was measured. Determination of TBA-reactive substances (TBARS), which was a complex between malondialdehyde (MDA) and thiobarbituric acid (TBA) was used as an index of the extent of lipid peroxidation according to the modified method of Esterbauer *et al*. (1991). The concentrations of TBA-reactive substances (TBARS) were calculated using Malondialdehyde bis (diethyl acetal) as a standard. The results were expressed as nmol MDA per milligram of protein.

7. Measurement of superoxide dismutase (SOD) enzymes activity

The SOD activity of homogenized brain hippocampus was measured using Cayman's superoxide dismutase assay kit (Cayman, USA). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of superoxide radical.

8. Statistical analysis

The data were presented as means±SE. Statistical significance was examined through one-way analysis of variance and Tukey tests, in which $p< 0.05$ was taken to be statistically significant.

RESULTS

1. Effect of different diets on brain hippocampus MDA content in CCl_4-treated rats

The result, as shown in Figure 1, revealed a significant increase of brain MDA level form rats in CCl_4 group compared with the control (p<0.05). Moreover, when compared to CCl_4 group, WR+CCl_4 and BR+CCl_4 group were not significantly different in MDA level (p<0.05) whereas PGBR+CCl_4 group showed a significant reduction of MDA level (p<0.05). Interestingly, PGBR+CCl_4 group was not significantly different in MDA level as compared to the control (p<0.05).

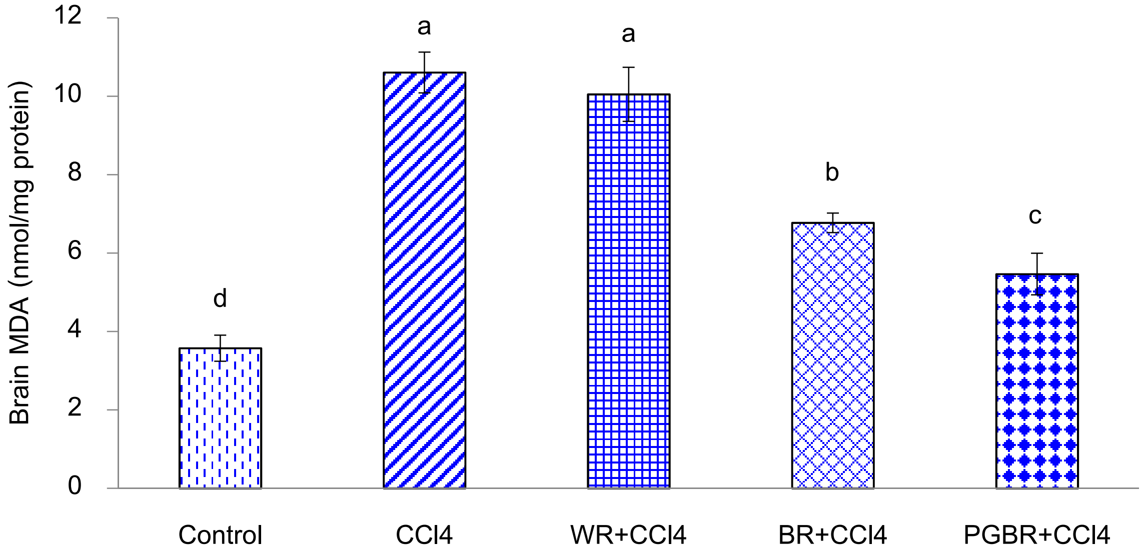

Figure 1 Effect of different diets on brain hippocampus MDA in CCl_4-treated rats. Data are presented as means±SE. Means with different superscripts (a, b, c, d) in each bar are significantly different (p< 0.05).

2. **Effect of different diets on brain hippocampus SOD activity in CCl_4-treated rats**

A significant decrease of SOD activity in rat brain compared to the control group was observed in CCl_4, WR+CCl_4 and BR+CCl_4 group (p<0.05) whereas PGBR+CCl_4 group was not significantly different in SOD activity compared to the control group (p<0.05) and significantly higher SOD activity than WR+CCl_4, BR+CCl_4 and CCl_4 group, as shown in Figure 2.

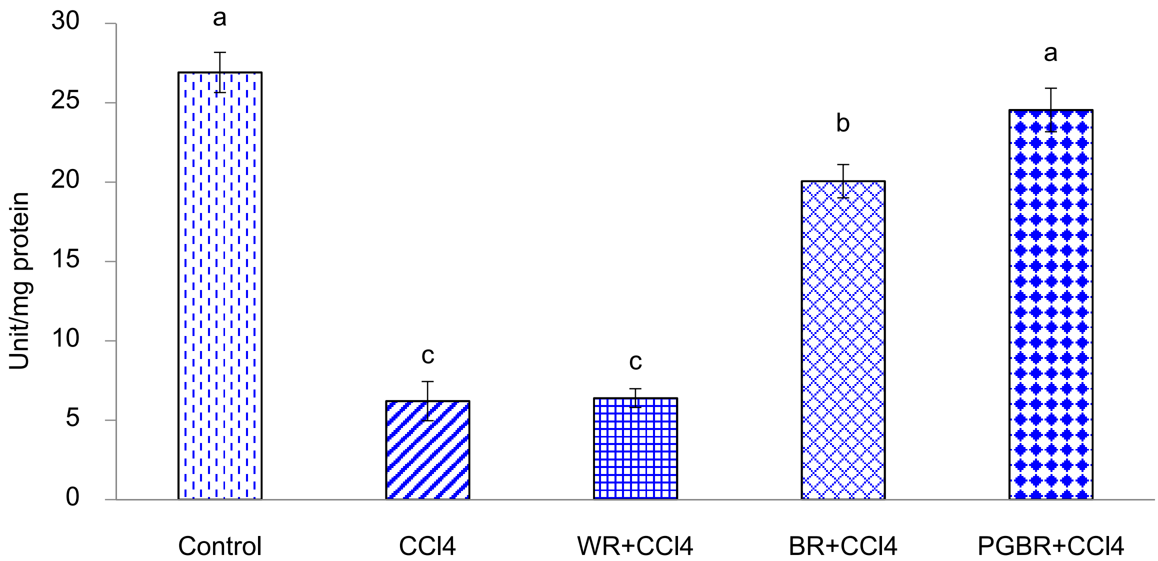

Figure 2 Effect of different diets on brain hippocampus SOD activity in CCl_4-treated rats. Data are presented as means±SE. Means with different superscripts (a, b, c) in each bar are significantly different (p< 0.05).

DISCUSSION

The previous studies indicated that CCl_4, induced rat brain oxidative stress by increasing lipid peroxidation and diminishing antioxidant enzyme activities and GSH content (Dani *et al*., 2008; Makni *et al*., 2012). Correspond to the present study, we found that the increase of MDA level and the decrease of SOD activity in brain hippocampus were observed in CCl_4-treated rats. Notwithstanding, the results of this study showed that rats fed with PGBR diet significantly alleviated brain hippocampus oxidative stress of rats caused by CCl_4. These findings may correspond to the abundant bioactive compounds found in PGBR, such as, ferulic acid, *p*-coumaric acid, γ-oryzanol and γ-tocotrienol, which were present at higher concentration in PGBR than in white rice and brown rice (Wunjuntuk *et al*., 2016b). Several studies have reported that these compounds have a potential to act as antioxidant which may suppress free radicals generated in brain hippocampus of rats induced by CCl_4 (Zhang *et al*., 2010; Ismail *et al*., 2012).

GABA, which was dramatically increased during germination of PGBR, may regulate the glutamatergic system by enhancing glutamate release and/or the sensitivity of NMDA receptors, resulting in memory enhancement (Zhang *et al*., 2010). Previous study of Hou (2011) reported that GABA prevented kainic acid (KA)-induced neuronal injury by suppressing lipid peroxidation in male FVB mice brain and inhibiting ROS accumulation in rat pheochromacytoma cell line (PC12). Shilpa *et al*. (2014) revealed that treatment of GABA and serotonin (5-HT) chitosan nanoparticles in partially hepatectomised rats were able to protect the neurons from ROS mediated cell damage and promoted their survival in the cerebral cortex *via* the regulation of gene expression of NF-κB, TNF-α, Akt-1 and SOD.

Ferulic acid exhibited the protection of neuronal cells against focal cerebral ischemic injury through its anti-oxidative and anti-inflammatory effects (Cheng *et al*., 2008). Moreover, Zhang *et al*. (2010) have demonstrated that pre-germinated brown rice (PR) improved learning and memory deficits induced by low-level lead (Pb) in young rats *via* several mechanisms: (1) the anti-oxidant activities of phenolic compounds featured reducing the ROS and MDA levels in the hippocampus and elevating the activities of SOD and GPx; (2) the high levels of GABA in PR might result in memory enhancement; and (3) PR diets might prevent Pb accumulation to some degree. Ismail *et al*. (2012) indicated that germinated brown rice (GBR) exhibited better neuroprotective and antioxidative activities than brown rice *via* free radical scavenging, chain-breaking and ferrous ions chelating actions in human neuroblastoma SH-SY5Y cells which were induced by H_2O_2. Therefore, PGBR may ameliorate CCl_4 induced oxidative damage to brain hippocampus by regulation of the balance between free radical production and antioxidant defenses.

CONCLUSION

These findings revealed that the experimental animal model of CCl_4-induced brain oxidative stress, rat-fed with PGBR-mixed diet could prevent brain hippocampus damage *via* the improvement of SOD activity and suppression of lipid peroxidation. This finding suggests that the regular consumption of PGBR can help to prevent chronic brain diseases associated with oxidative stress.

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