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Red Mold Rice against Hepatic Inflammatory Damage in Zn-deficient Rats

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Abstract

The protective effect of red mold rice (RMR) against liver injury in rats fed with a Zn-deficient diet for 12 weeks was investigated in this study. Rats were orally administered RMR (151 mg/kg body weight or 755 mg/kg body weight; 1 × dose or 5 × dose, respectively) with or without Zn once a day for 4 consecutive weeks. The severity of liver damage was evaluated by measuring the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in Zn-deficient rats. RMR significantly inhibited the elevation of serum ALT levels by Zn-deficient induction. Hepatic antioxidase activity was also significantly increased in the RMR + Zn group (RZ), thereby suppressing the productions of reactive oxygen species (ROS) and proinflammatory cytokines in the liver of Zn-deficient rats. These findings suggested that RMR exerted hepatoprotective effects against Zn deficiency-induced liver inflammation.

Key words: Red mold rice (RMR); Liver injury; Zn-deficient diet; Antioxidase activity; Proinflammatory cytokines

Introduction

Red mold rice (RMR) (紅麴, hóng qū) contains a large amount of monacolins, which as an inhibitor of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Furthermore, RMR contains various antioxidants such as dimerumic acid, tannin, and phenol (Lee et al., 2006). In our previous study, RMR has shown antioxidative abilities, including reducing power and 1,1-diphenyl-2-pichrylhydrazyl (DPPH) radical scavenging activity (Lee et al., 2007), and antioxidase-stimulating activity in the brain of Zn-deficient rats (Lee et al., 2009; Lee et al., 2011a). Red meat and several foods including seafood, cereals, and nuts are common sources of Zn, but they contain a good deal of cholesterol, Zn inhibitors, and calorific capacity.

As the mammalian body does not store Zn; therefore, a constant dietary intake of this micronutrient is essential (Prasad et al., 1996). According to the 1989 US guidelines, the recommended daily allowances (RDA) of Zn are as follows: 5 mg/day for infants; 10 mg/day for children; 15 mg/day for teenagers, adults, and pregnant women; and 16-19 mg/day for lactating women (Solomons, 1986). The study indicates that Zn can potentially stimulate antioxidase activity, including catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and superoxide dismutase (SOD), thereby inhibiting hepatic lipid peroxidation (Goel et al., 2005). Stabilization of CAT activity has been reported to dependent on Zn, because this protein contains four cysteine residues in each subunit thereby binding to Zn to prevent a decrease in catalase activity caused by reactive oxygen species (ROS) (Gantchev and Van-Lier, 1995; Tate et al., 1999). An epidemiological study has shown that a diet containing antioxidants can lower the risk of diseases associated with oxidative stress in humans (Ness and Powles, 1997).

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Occurrence of Zn deficiency is always found in elder worldwide (Prasad et al., 1996). Low intracellular Zn is associated with aging and pathological conditions such as diabetes mellitus, memory impairment, and sperm injury (Barthel et al., 2007; Lee et al., 2009; Lee et al., 2011a). In addition, Zn deficiency results in an increase in proinflammatory cytokine and a decrease in metallothionein homeostasis (Fabris and Mocchegiani, 1995). Zn deficiency is also involved in chronic inflammation (Zhou et al., 2004). Study has demonstrated that Zn exerts hepatoprotection by inhibiting collagen accumulation in liver (Dhawan and Goel, 1995). A study also supports the concept that oxidative stress is an underlying factor of inflammatory responses for triggering hepatocyte apoptosis (Sunderman, 1995), suggesting that hepatocyte apoptosis and ROS overproductions are observable in the case of long-term Zn deficiency (Nodera et al., 2001).

Materials and methods

Chemicals

The C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CRE) and SOD assay kits were purchased from Randox Laboratories Ltd. (Antrim, United Kingdom). Glutathione (GSH), GR, 5,50-dithiobis-2-nitrobenzoic acid (DTNB), glutathione disulfide (GSSG), nicotinamide adenine dinucleotide phosphate (NADPH), and nitroblue tetrazolium (NBT) were purchased from Sigma Co. (St. Louis, MO, USA).

Sample preparation

Red mold rice (RMR) is obtained from *Monascus purpureus* NTU 568 fermented rice. *Monascus purpureus* NTU 568 strain was maintained on potato dextrose agar (PDA) slanted at 4℃and transferred monthly. The preparation of RMR was carried out under the substrate of long-grain rice (Oryza sativa) purchased from a local supermarket in Taiwan and using the method of solid-state culture. Briefly, a 500 g of rice was soaked in distilled water for 8 h. After that, excess water was removed with a sieve. The rice was autoclaved with autoclave (HL-341 model, Gemmy Corp, Taipei, Taiwan) for 20 min at 121℃in a “koji-dish” (the koji-dish was made of wood with the dimensions of 30×20×5 cm) that is a fermented instrument tray of RMR during the fermentation process. After having been cooled, the rice was inoculated with a 5% (v/w) spore suspension. The inoculated rice was cultivated at 30℃ for 10 days. During the culturing stage, 100 mL of water was daily added to the rice from the second day to the fifth day. At the end of cultivation, the crushed and dried product with the mold was used for the experiments.

Animal and diets

Male Wistar rats (4-week old) (90.7 ± 10.6 g) were obtained from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Animals were acclimatized for 1 week prior to use, they were divided at random into seven treatment groups (9 rats per group) and provided with food and water ad libitum. Animals were subjected to 12 h light/dark cycle with a maintained relative humidity of 60% and a temperature at 25 ℃ (Protocol complied with guidelines described in the “Animal Protection Law”, amended on Jan. 17, 2001 Hua-Zong-(1)-Yi-Tzi-9000007530, Council of Agriculture, Executive Yuan, Taiwan, ROC). Zn-deficient diet was provided in accordance with the formula (Shaheen and Abd El-Fattah, 1995) that containing 200.0 g egg white, 631.1 g dextrose, 100.0 g corn oil, 30.0 g fiber, 9.9 g calcium carbonate, 3.2 g calcium phosphate, 0.002 g cobalt chloride, 0.01 g cupric sulphate, 0.9 g ferric citrate, 3.4 g magnesium sulphate, 0.009 g manganese sulphate, 0.026 g potassium iodide, 5.55 g sodium chloride, 0.004 g biotin, 0.02 g vitamin B_{12}, 0.016 g calcium pantothenate, 1.5 g choline chloride, 0.25 g chlorotetracycline, 0.0005 g folic acid, 0.0003 g menadione, 0.25 g niacin, 0.004 g pyridoxine•HCl, 0.006 g riboflavin, 0.01 g thiamin•HCl, 10000 IU retinyl palmitate, 1250 IU ergocalciferol, and 110 IU tocopheryl acetate/kg diet. Rats were fed with the daily diet containing 60 mg/kg of Zn in the control group, but only approximate 0.3 mg/kg of Zn was found in Zn-deficient feedstuff. All animals were divided into 2 groups (normal and Zn-deficient) during the first 12 weeks, and the Zn-deficient rats were later divided into six groups that included Zn-deficient (ZD), Zn-compensation (ZC), 1 × RMR (1R), 5 × RMR (5R), 1 × RMR + Zn (1RZ) and 5 × RMR + Zn (5RZ) groups, respectively. Animals were induced to be Zn deficiency by the Zn-deficient diet for 12 weeks prior to 4 weeks of sample administration.

Dose and grouping

Rats were divided at random into the seven groups, including normal, ZD, ZC, 1R, 5R, 1RZ and 5RZ groups. Rats were administered sample per-oral (po) and the dosage of RMR was calculated in accordance with

Body's formula of body surface area as recommended by the Food and Drug Administration (FDA) (Boyd, 1935). The daily dietary dosage of RMR is usually recommended at 1.0-2.0 g for adults (Heber et al., 1999). Therefore, 2 g of RMR was used as the 1-fold dosage for an adult with a weight of 65 kg and a height of 170 cm, and 151 mg/kg body weight (bw) (1-fold dosage) of RMR was used as a frame of reference for conversion of the dosage into a rat model, and 755 mg/kg bw of RMR was used as 5-fold dosage, relatively. We have known that RMR (755 mg/kg bw) significantly promoted antioxidase activity in brain and testes of Zn-deficient rats (Lee et al., 2009; Lee et al., 2011a). Based on 1989 USA guidelines, the recommended dietary allowances (RDA) of Zn are 5 mg/day for infants, 10 mg/day for children, 15 mg/day for teenagers, adults, and pregnant women, and 16-19 mg/day for lactation women. On the other hand, the level of Zn in RMR was 25.9 mg/kg. Thus, the administration level of 1R (151 mg/kg bw) contained 0.004 mg of Zn/kg bw, and 0.02 mg of Zn/kg bw was administered in the 5R group. However, the Zn gluconate was employed to compensate in the ZC group (the administration level of Zn was 1.1 mg/kg bw), which was in accord with the RDA. Accordingly, a level of 0.004 + 1.1 mg Zn/kg bw was administered in the 1RZ group, and a level of 0.02 + 1.1 mg Zn/kg bw was administered in the 5RZ group.

Assay for antioxidant enzymes

The animals were sacrificed with CO_2 asphyxia. The liver and blood were then removed. Blood were placed at room temperature for 1 h, and then centrifuged at 1000*g* for 10 min to obtain serum. Livers were respectively homogenized in ice-cold 20 mM Tris-HCl (pH 7.4) (1:10, w/v). The homogenates were centrifuged (12000*g*) for 30 min at 4 ℃ and stored at -80 ℃ for the following experiments. Glutathione peroxidase (GPx) activity was determined as previously described (Mohandas et al., 1984). Briefly, 0.1 mL of homogenate was mixed with 0.8 mL of 100 mM potassium phosphate buffer (1 mM EDTA, 1 mM NaN3, 0.2 mM NADPH, 1 unit/mL GR, and 1 mM GSH, pH 7.0) and incubated for 5 min at room temperature. Thereafter, the reaction was initiated after the adding of 0.1 mL of 2.5 mM hydrogen peroxide (H_2O_2). GPx activity was calculated by the change of the absorbance at 340 nm for 5 min. In another reaction containing 0.1 M phosphate buffer (1 mM $MgCl_2$•$6H_2O$, 50 mM GSSG, and 0.1 mM NADPH, pH 7.0) was added 0.1 mL of homogenate for glutathione reductase (GR) activity determination, and the decreased absorbance at 340 nm was measured for 3 min (Bellomo et al., 1987). The catalase (CAT) activity was determined according to the method of Aebi (1984). Fifty microliters of homogenate was mixed with 950 μL of 0.02 M H_2O_2 and incubated at room temperature for 2 min. CAT activity was calculated by the change of the absorbance at 240 nm for 3 min. The assay of SOD activity was accomplished with a commercial kit (Randox Laboratories Ltd., Antrim, United Kingdom).

Assays for GSSG and ROS

The GSSG content of liver homogenate was determined as previous description (Van-Dam et al., 1999). Liver homogenate was mixed with 5% cold trichloroacetic acid (TCA) mixture and incubated for 5 min, centrifuged at 8000g for 10 min at 4 ℃. The homogenate was reacted with 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) for 5 min at 4 ℃. The concentration of GSSG was calculated and expressed by μmole/mg protein. The ROS level was measured by nitroblue tetrazolium (NBT) reaction. NBT is reduced to form blue-black formazan by ROS. Briefly, 100 μL of homogenates was added into 96-well plates, and 10 mg/mL of NBT was added and incubated for 1 h, and the absorbance was measured at 570 nm.

Assay for caspase-3, caspase - 8, and caspase -9

The caspase-3, caspase-8, and caspase-9 activities of liver homogenate were determined with kits from BioVision Inc. (Mountain View, CA, USA). The homogenate was added 50 μL of 2X reaction buffer (contained 10 mM dithiothreitol), and mixed with 5 μL of 4 mM substrate (200 μM to final concentration) for 1-2 h incubation (37 ℃). Read optical density of samples at 405 nm in a microliter plate reader.

Assay for cytokines

The IL-6, TNF-α and IL-1β levels of liver homogenate and serum were determined with ELISA kits from Peprotech (Rocky Hill, NJ, USA .

Statistical analysis

Above data are expressed as means ± SD. The software of ANOVA was used to evaluate the difference between multiple groups. If significance was observed between each group, Duncan's multiple range was used to compare the means of two specific groups. And $P < 0.05$ was considered to be significant.

Table 1. The effects of RMR on serum biochemical values

Groups	AST	ALT	BUN	CRE
	unit/L		mg/dL	
Normal	95.9 ± 13.5	43.7 ± 10.8	17.6 ± 2.7	0.4 ± 0.1
ZD	100.4 ± 9.9	80.2 ± 10.9*	33.3 ± 6.1*	0.4 ± 0.1
ZC	102.8 ± 11.9	53.4 ± 11.2#	23.0 ± 5.3	0.5 ± 0.2
1R	128.0 ± 4.1	51.5 ± 15.2#	26.9 ± 3.1	0.5 ± 0.2
5R	103.1 ± 19.3	40.5 ± 5.2#	25.7 ± 6.4	0.5 ± 0.1
1RZ	111.6 ± 31.8	60.8 ± 6.2#	21.5 ± 8.8#	0.4 ± 0.2
5RZ	101.6 ± 39.2	43.0 ± 14.3#	18.1 ± 4.6#	0.5 ± 0.1

Each value is expressed as mean ± SD (n = 9). Rats were divided into the normal, Zn-deficient (ZD), Zn-compensative (ZC), 1× RMR (1R), 5× RMR (5R), 1× RMR + Zn (1RZ) and 5× RMR + Zn (5RZ) groups. AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen, CRE: creatinine. *Significantly different ($p < 0.05$) vs. the normal group. #Significantly different ($p < 0.05$) vs. the Zn-deficient group.

Results

Effects of RMR on serum biochemical values

Zn deficiency can result in liver and kidney damage (Nodera et al., 2001). The serum biochemical results indicated that liver was damaged causing by Zn deficiency induction (Table 1). Serum ALT and BUN levels in the ZD group were significantly elevated comparing to the normal group. However, RMR (1× and 5× doses) with or without Zn administration markedly inhibited these rises in serum ALT and BUN of ZD rats. These observations implied that RMR exerted protection against Zn deficiency-induced liver damage.

Effects of RMR on antioxidant enzyme activity

In the ZD group, a notable decrease was observed in hepatic CAT, GR, GPx, and SOD activities (Table 2), with a subsequently resulting in increases in hepatic ROS and GSSG (Figure 1). Zn compensation recovered these effects for up-regulating hepatic CAT, GR, GPx, and SOD in the ZC group comparing to the ZD group. The elevations of hepatic CAT, GR, and GPx by 5RZ were greater than ZC administration.

The Zn-deficient diet has shown to increase hepatic ROS and GSSG (Figure 1); these increases would result in liver dysfunction, which was confirmed by serum ALT level (Table 1). ROS and GSSG were notably lowered in the 5R, 1RZ, and 5RZ groups, indicating that Zn deficiency increased ROS production and GSH oxidation to form GSSG. However, RMR and Zn exerted antioxidative ability to recover hepatic GSH and suppress GSSG levels. *Monascus* production contains several antioxidant and anti-inflammatory pigments such

Table 2. The effects of RMR on hepatic antioxidant enzyme activity of zinc-deficient rats

Groups	CAT	GR	GPx	SOD
	nmol H_2O_2/min/mg protein	nmol NADPH/min/mg protein		unit/mg protein
Normal	49.6 ± 7.1	216.0 ± 7.0	1492.6 ± 158.1	6.0 ± 0.3
ZD	32.2 ± 3.9*	85.0 ± 8.7*	1162.1 ± 138.7*	4.7 ± 0.5*
ZC	42.6 ± 6.4	134.7 ± 9.2#	1601.8 ± 144.4#	5.5 ± 0.3
1R	37.8 ± 2.4	103.1 ± 4.6#	1650.1 ± 129.6#	5.5 ± 0.2
5R	69.6 ± 5.0#	128.9 ± 4.4#	1820.2 ± 105.3#	5.7 ± 1.1#
1RZ	57.2 ± 3.5#	158.3 ± 7.2#	1750.7 ± 117.9#	5.7 ± 0.6#
5RZ	99.2 ± 16.0#	200.0 ± 5.9#	1889.5 ± 137.1#	5.8 ± 0.4#

Each value is expressed as mean ± SD (n = 9). Rats were divided into the normal, Zn-deficient (ZD), Zn-compensative (ZC), 1× RMR (1R), 5× RMR (5R), 1× RMR + Zn (1RZ) and 5× RMR + Zn (5RZ). CAT: catalase; GR: glutathione reductase; GPx: glutathione peroxidase; SOD: superoxide dismutase; ROS: reactive oxygen species. *Significantly different from the normal group, $p < 0.05$. #Significantly different from the ZD group, $p < 0.05$.

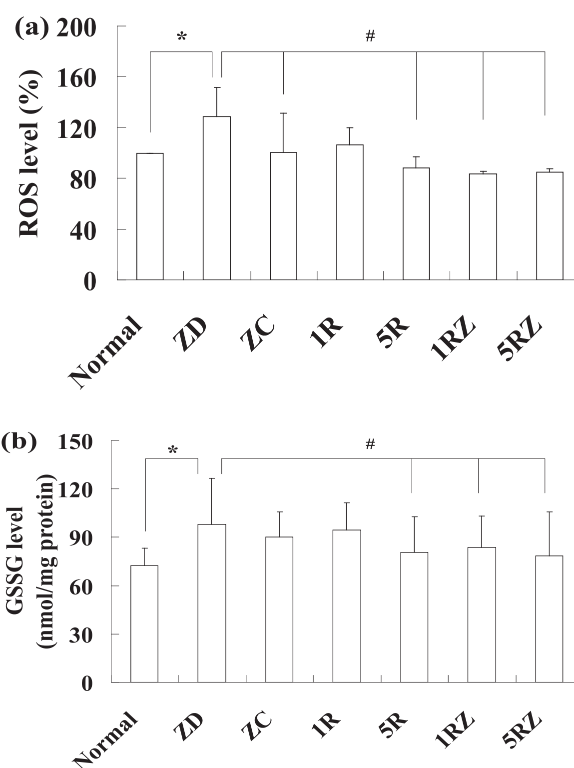

Figure 1. The effects of RMR on ROS (a) and GSSG (b) in the liver of zinc-deficient rats. Each value is expressed as mean ± SD (n = 9). Rats were divided into the normal, zinc-deficient (ZD), zinc-compensative (ZC), 1× RMR (1R), 5× RMR (5R), 1× RMR + zinc (1RZ) and 5× RMR + zinc (5RZ). *Significantly different from the normal group, $p < 0.05$. #Significantly different from the ZD group, $p < 0.05$.

as monascin and ankaflavin, which were respectively presented as 2177.3 mg/kg and 3444.2 mg/kg in RMR (Lee et al., 2009). These findings indicated that RMR displayed anti-inflammatory and antioxidant roles in the Zn-deficient rats.

Effects of RMR on caspase-3, caspase-8, and caspase-9

There are two apoptosis signaling pathways associated with different caspases. One pathway is initiated by the Fas/death or TNF-α receptor. In the Fas/death signaling pathway, Fas activation promotes Fas-associated death domain (FADD) activity via an interaction between Fas and FADD. Subsequently, procaspase-8 binds to Fas-FADD complex following activation. Caspase-8 in turn activates caspase-3 to inducing apoptosis. The other pathway is regulated by the release of cytochrome c from mitochondria, resulting in caspase-9 and caspase-3 activation. Depletion of available Zn in the liver would lead to caspase-3 activation and apoptosis (Truong-Tran et al., 2000; Truong-Tran et al., 2002).

As shown in Figure 2, results found that the absence of caspase-3 activity and no notable up-regulation for hepatic caspase-9 activity of ZD-rats, suggesting that hepatocytes was not induced apoptosis by 16 weeks of Zn deficiency. The histopathological examination revealed without liver injury in Zn-deficient rats, whereas hepatocyte apoptosis is induced by of Zn deficiency (34 weeks) (Nodera et al., 2001). Although Zn level (25.1 mg/kg) was lowered in the liver of ZD rats comparing to the normal group (39.4 mg/kg), but the liver of ZD rats remained bits of Zn (Table 3); thus,

Table 3. The effects of RMR on the trace element levels of liver

Groups	Fe	Zn	Ca	Mg
	Concentration (mg/kg)			
Normal	248.3 ± 27.8	39.4 ± 5.0	40.1 ± 2.8	136.4 ± 11.4
ZD	446.8 ± 68.7*	25.1 ± 2.5*	54.6 ± 11.1*	147.2 ± 3.0
ZC	368.8 ± 77.5	33.3 ± 1.7#	39.8 ± 4.0#	144.2 ± 1.4
1R	407.4 ± 82.8	27.8 ± 3.6	47.0 ± 3.3	140.5 ± 6.8
5R	383.9 ± 35.1#	30.6 ± 2.5	43.3 ± 3.6	140.8 ± 5.6
1RZ	344.6 ± 63.2#	49.6 ± 10.1#	40.1 ± 2.8#	135.3 ± 16.1
5RZ	323.8 ± 37.1#	57.0 ± 2.4#	35.2 ± 4.3#	138.5 ± 7.4

Each value is expressed as mean ± SD (n=9). Rats were divided into the normal, Zn-deficient (ZD), Zn-compensative (ZC), 1× RMR (1R), 5× RMR (5R), 1× RMR + Zn (1RZ) and 5× RMR + Zn (5RZ). *Significantly different from the normal group, $p < 0.05$. #Significantly different from the ZD group, $p < 0.05$.

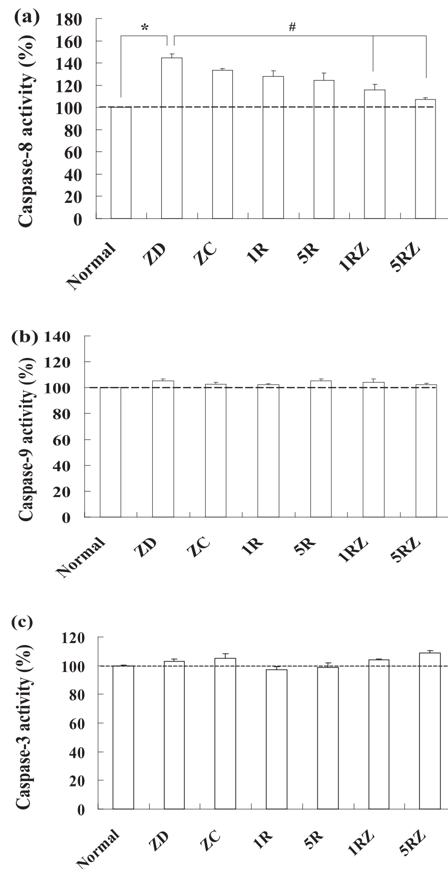

Figure 2. The effects of RMR on caspase-8 (a), caspase-9 (b), and caspase-3 (c) in the liver of zinc-deficient rats. Each value is expressed as mean ± SD (n = 9). Rats were divided into the normal, zinc-deficient (ZD), zinc-compensative (ZC), 1× RMR (1R), 5× RMR (5R), 1× RMR + zinc (1RZ) and 5× RMR + zinc (5RZ). *Significantly different from the normal group, $p < 0.05$. #Significantly different from the ZD group, $p < 0.05$.

hepatocytes apoptosis was not found in the ZD-rats. Furthermore, Zn deficiency-induced elevation of hepatic Ca and Fe was toxic for liver. Administration of 1R and 5R both alleviated these rises.

In addition, hepatic caspase-8 activity was significantly elevated in the ZD rats (Figure 2); this finding was intriguing because caspase-3 activation and apoptosis both were not observed in the liver of ZD rats. Therefore, we in turn investigated that caspase-8 activation whether participating pro-inflammation in ZD inducing liver damage.

Inflammatory factors

C-reactive protein (CRP) is abundantly produced during liver inflammation. Although Zn deficiency increased the serum CRP levels (Beattie et al., 2006), but RMR effectively lowered this elevation (Figure 3). This finding indicated that RMR can inhibit inflammation caused by Zn-deficient induction.

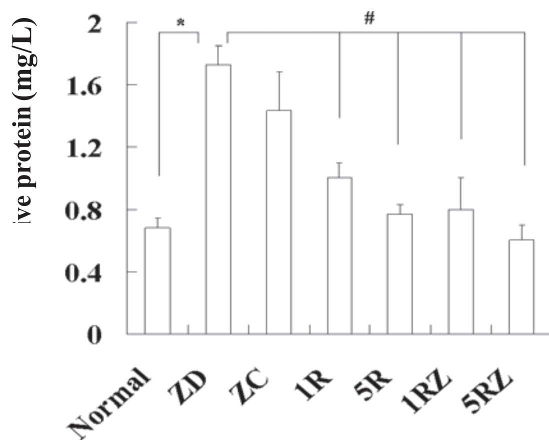

Figure 3. The effects of RMR on C-reactive protein in the serum of ZD-rats. Each value is expressed as mean ± SD (n = 9). Rats were divided into the normal, zinc-deficient (ZD), zinc-compensative (ZC), 1× RMR (1R), 5× RMR (5R), 1× RMR + zinc (1RZ) and 5× RMR + zinc (5RZ). *Significantly different from the normal group, $p < 0.05$. #Significantly different from the ZD group, $p < 0.05$.

Caspase-8 and caspase-1 both has been shown to play a proinflammatory role in increasing IL-1β level and maturation (Maelfait et al., 2008). Zn deficiency leads to the secretion of inflammatory cytokines (IL-1β, TNF-α, and IL-6), which subsequently resulting in oxidative stress (Prasad et al., 2007; Liuzzi et al., 2005). Serum and hepatic TNF-α, IL-1β, and IL-6 were significantly increased in the ZD group (Figure 4). However, 5R and 5RZ markedly suppressed increases in serum and hepatic TNF-α, IL-1β, and IL-6 strongly than ZC administration, and these findings were contributed to RMR attenuate caspase-8 activity.

Discussion

Zn deficiency can lead to many diseases, including liver inflammation. Chronic liver inflammation is associated with the aging process because continuous depletion of intracellular Zn. In this study, Zn (0.004 mg/kg bw and 0.02 mg/kg bw for 1R and 5R groups, respectively) was compensated in RMR administering groups. These dosages were lower than ZC administration (1.1 mg/kg bw). However, 1R and 5R treatment both improved hepatic inflammatory damage than ZC administration, suggesting that RMR could exert hepatoprotection.

Oxidative stress has been implicated as one important factor for organ injury and inflammation (Racasan et al., 2004; Ishikawa et al., 2006; Pearson et al., 2001), and results in malondialdehyde (MDA) production, an index of lipid peroxidation (Landmesser et al., 2002). ROS can active mitogen-activated protein kinase (MAPK) phosphorylation to result in transcription factor activation and inflammatory factor production (Zhang et al., 2007). Furthermore, suppression of MAPK can effectively attenuate organ dysfunction and inhibit inflammatory reaction.

The mechanism of Zn deficiency-induced oxidative damage in rats is investigated (Kim and Keen, 1999; Prasad et al., 2007). Zn potentially maintains antioxidase acitivty (Cu/Zn SOD) and inhibits lipid peroxidation and inflammation in the liver (Goel et al., 2005; Ozaras et al., 2003). A previous study has shown that Zn reduces collagen accumulation (Goel and Dhawan, 2005) and prevents free radicals production in the liver to induce liver damage (Nodera et al., 2001). Our findings indicated that RMR could improve damage caused by Zn-deficient induction (Table 1). However, caspase-8 activity was notably increased in the liver of the Zn-deficient rats. In addition, TNF-α may block the death signaling pathway in hepatocyte apoptosis mediating by caspase-8 (Nagaki et al., 2000). Moreover, caspase-1 composes inflammasome which is initiator in inflammation pathway to result in IL-1β maturation; however, study has reported that caspase-8 can activate caspase-1 (Ganz et al., 2011; Maelfait et al., 2008). We believed that caspase-8 played an important proinflammatory role in promoting IL-1β level and maturation by activating caspase-1 (Figures 2 and 4), albeit caspase-3 and caspase-9 both were not activated in the ZD group.

Recently, we demonstrate that *Monascus*-fermented products exert antioxidative and anti-inflammatory activities (Shi and Pan, 2010; Hsu et al., 2010). We hypothesized three anti-inflammatory mechanisms by RMR administration in ZD rats: (1) RMR may indirectly inhibits increases in caspase-8 and IL-1β caused by Zn deficiency; (2) RMR reduces TNF-α production to avoid TNF-α signaling interfering caspase-8 activity in the liver; and (3) RMR suppressed TNF-αT level to attenuate Zn deficiency inducing hepatocyte apoptosis, in turn alleviating TNF-α-activated caspase-8 and reducing IL-1β production.

Monascus-fermented products such as monascin and ankaflavin have been reported to show anti-inflammatory activity in our recent studies (Lee et al., 2011b; Hsu et al., 2010). *Monascus*-fermented product contains anti-inflammatory pigments, including monascin and ankaflavin, which were respectively presented as 2177.3 mg/kg and 3444.2 mg/kg in RMR, to administrate to ZD rats (Lee et al., 2009), suggesting that monascin and ankaflavin may exert inhibitory

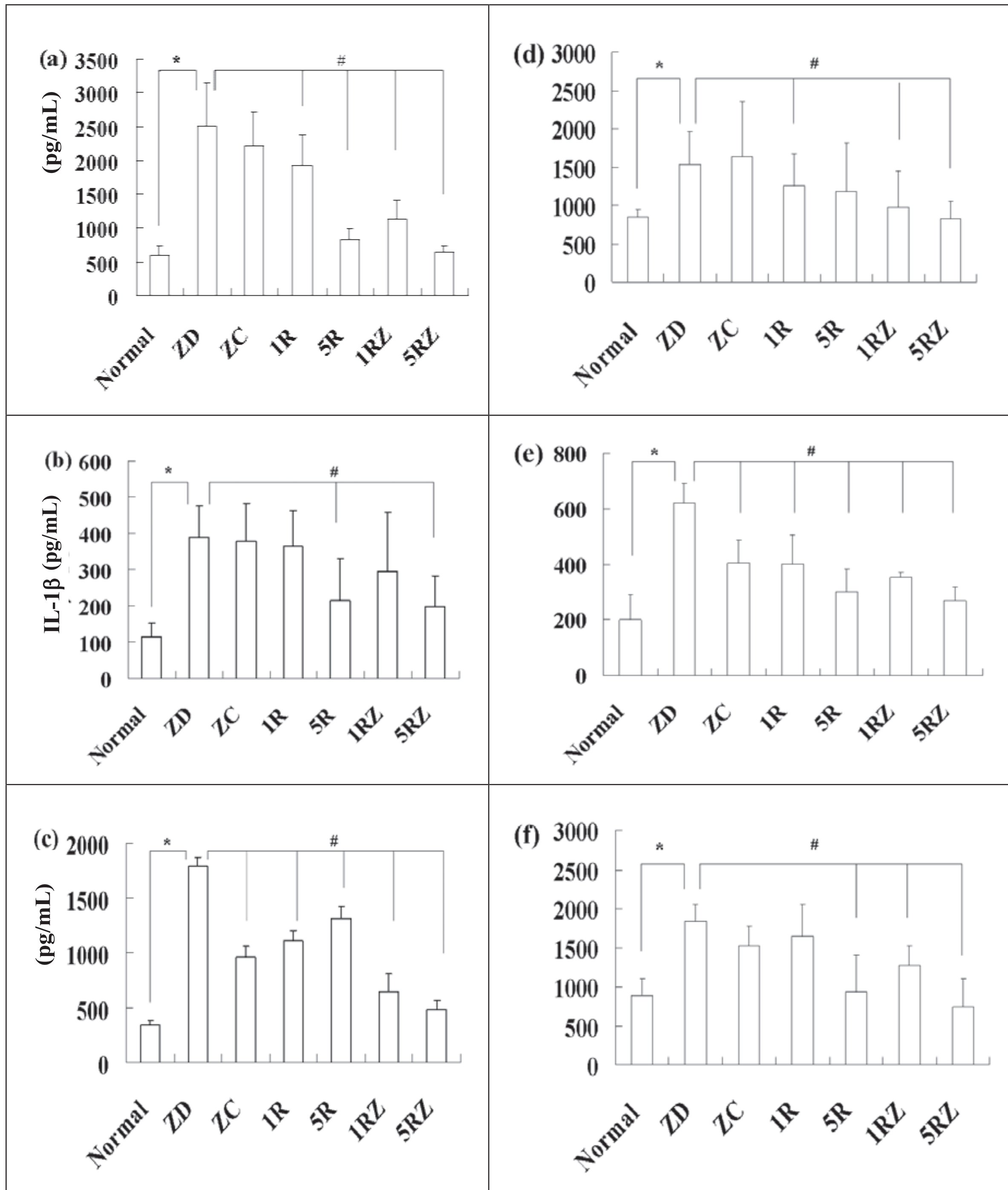

Figure 4. The inhibitive effects of RMR on inflammatory factors in the serum (a-c) and liver (d-f) of zinc-deficient rats. Each value is expressed as mean ± SD (n = 9). Rats were divided into the normal, zinc-deficient (ZD), zinc-compensative (ZC), 1× RMR (1R), 5× RMR (5R), 1× RMR + zinc (1RZ) and 5× RMR + zinc (5RZ). *Significantly different from the normal group, $p < 0.05$. #Significantly different from the ZD group, $p < 0.05$.

activity of inflammation in the ZD rats. These findings indicated that RMR displayed anti-inflammatory and antioxidant roles in the Zn-deficient rats. Overall, RMR has been used for many centuries to enhance the color and flavor of food, as well as a traditional medicine for digestive and vascular functions (Journoud and Jones, 2004). Zn deficiency is always occurring to elder to result in hepatic inflammatory damage, including elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum. However, RMR has been reported to inhibit inflammation and oxidative stress in brain and testis of Zn-deficient in our previous studies (Lee et al., 2009; Lee et al., 2011a). In conclusion, we hypothesized that the hepatoprotection of RMR through suppressed oxidative stress against liver inflammation by Zn-deficient induction.

Conflict of Interest

This research work and subsidiary spending were supported by Paolyta Co., Ltd (Taipei, Taiwan).

Abbreviations

Red mold rice (RMR); Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Reactive oxygen species (ROS); Catalase (CAT); Glutathione reductase (GR); Glutathione peroxidase (GPx); Glutathione-S-transferase (GST); Superoxide dismutase (SOD); C-reactive protein (CRP); Blood urea nitrogen (BUN); Creatinine (CRE)

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