

*Full Length Research Paper*

# High levels of 25-hydroxyvitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] and $\alpha$ -tocopherol prevent oxidative stress in rats that consume Thai brown rice

Prasit Suwannalert<sup>1\*</sup>, Sirichet Rattanachitthawat<sup>2</sup>, Chaiyavat Chaiyasut<sup>3</sup> and Suda Riengrojpitak<sup>1</sup>

<sup>1</sup>Department of Pathobiology, Faculty of Science, Mahidol University, Rama 6 Road, Phayathai, Ratchathewi, Bangkok 10400, Thailand.

<sup>2</sup>Faculty of Agricultural Technology, Burapha University Sakaeo Campus, Sakaeo 27160, Thailand.

<sup>3</sup>Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.

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**Oxidative stress has been proposed to play an important role in the progression of chronic diseases. The red color strain of Thai brown rice, a high source of phenolic compounds, may play a crucial role in oxidative stress prevention. In the present study, rats were fed with 0% (Control), 10 and 70% Thai brown rice in the mixed food. The serum malondialdehyde (MDA), ferric reducing antioxidant power (FRAP), storage vitamin D [25(OH)D<sub>3</sub>] and  $\alpha$ -tocopherol were investigated. The mean value of MDA in high and low dose groups was significantly lower than that of the controls in both male and female. FRAP in the high dose males was significantly higher than that in the control. Mean value of MDA and  $\alpha$ -tocopherol was inversely related ( $r = -0.538$ ,  $p = 0.001$ ). Interestingly, serum 25(OH)D<sub>3</sub> of the high dose group was more significant different than that of the controls in both males ( $p = 0.001$ ) and females ( $p = 0.005$ ). Moreover, MDA level was strongly inversely related to that of 25(OH)D<sub>3</sub> ( $r = -0.656$ ,  $p < 0.001$ ). The results indicated that the rats consuming Thai brown rice possessed low level of oxidative stress marker, MDA, through both radical and non radical defenses.**

**Key words:** Oxidative stress, radical, non radical, vitamin D,  $\alpha$ -tocopherol.

## INTRODUCTION

An imbalance between reactive oxygen and biological elimination system lead to oxidative stress (Buonocore and Groenendaal, 2007; Favier, 2006). Oxidative stress was implicated on cellular components, such as lipid, protein and DNA (Blokhuin et al., 2003; Imlay and Linn, 1988) which eventually caused cellular damage and developed clinical diseases including cardiovascular disease, diabetes, neurodegenerative disorders and aging (Favier, 2006; Halliwell, 1994; Halliwell et al., 1992; Newsholme et al., 2007). Dietary micronutrients which include ascorbic acid,  $\alpha$ -tocopherol, flavonoids and carotenoids

contribute to the antioxidant defense system (Dragsted, 2008; Tapiero et al., 2004). Although radical species had been studied widely as a mechanism of oxidative stress (Toyokuni and Akatsuka, 2007); oxidative stress could occur without free radical. Non radical oxidants could lead to disruption of redox signaling and control, anti-oxidative stress circuit (Jones, 2008). The thiol redox system requires glucose-6-phosphate dehydrogenase (G6PD) to drive the function. G6PD has been shown to be dose and time dependent following induction by the compound vitamin D (Bao et al., 2008; Jones, 2006; Kehrer and Lund, 1994).

Brown rice is a high source of tocopherol, tocotrienols, ferulic acid, oryzanols and phenolic compounds (Srinivasan, 2007; Tian et al., 2004). The phenolic component could raise serum vitamin D<sub>3</sub> (Wietrzyk,

\*Corresponding author. E-mail: [parasit109@yahoo.com](mailto:parasit109@yahoo.com). Tel: +66-2-201-5550. Fax: +66-2-354-7158.

2007). Thus, Thai brown rice supplementation may play a beneficial role in preventing oxidative stress through both radical and non radical mechanisms. In this study, serum oxidative stress marker (MDA), antioxidant marker (FRAP), 25(OH)D<sub>3</sub> and  $\alpha$ -tocopherol were investigated in rat models.

## MATERIALS AND METHODS

### Animals

Thirty six healthy male and female rats with mean body weight 460.53 and 258.88 g, respectively, were classified into 3 groups according to the percentage of red strain Thai brown rice mixed with the commercial standard food: control (0%), low dose (10%) and high dose (70%). Two months later, blood samples were collected from the rats by carotid-puncture and centrifuged at 3000 g for 15 min at room temperature. Serum oxidative stress marker, antioxidant marker, storage vitamin D and  $\alpha$ -tocopherol were investigated.

### Malondialdehyde (MDA)

The concentration of MDA based on the reaction of thiobarbituric acid (TBA) was determined by Suwannalert modified method (Suwannalert et al., 2007). Serum 100 and 50  $\mu$ l of 7.2% butylated hydroxytoluene (BHT) were mixed with 1.5 ml 25 nmol/L TBA, 1.5 ml HCl, 550  $\mu$ l distilled water, 200  $\mu$ l of 8.1% SDS and 50  $\mu$ l of 7.2% BHT. The reaction mixture was incubated at 90°C for 15 min and rapidly cooled for 10 min; then 0.5 ml of distilled water and 3 ml of n-butanol in pyridine were added to the reaction mixture. They were mixed vigorously and centrifuged at 3000 g for 15 min. The MDA product was measured by using a Multimode Detector (Breckman, DTX 880, Australia) at 520 nm excitation and 550 nm emission.

### Ferric reducing antioxidant power (FRAP)

The method based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe<sup>3+</sup>-TPTZ) to ferrous (Fe<sup>2+</sup>-TPTZ) was used. Working FRAP reagent was freshly prepared by mixing 25 ml acetate buffer pH 3.6, 2.5 ml of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ solution) and 2.5 ml of 20 mM ferric chloride hexahydrate (FeCl<sub>3</sub>.6H<sub>2</sub>O) solution. Serum sample was mixed with working FRAP reagent and incubated at room temperature for 10 min. The absorbance of ferrous complex determined by a multimode detector at 595 nm was compared to that of ferrous sulfate heptahydrate (FeSO<sub>4</sub>.7H<sub>2</sub>O) standard.

### 25(OH)D<sub>3</sub> and $\alpha$ -tocopherol levels

Serum 25(OH)D<sub>3</sub> and  $\alpha$ -tocopherol were determined according to the modified method of Turpeinen et al. (2003). Serum sample was mixed briefly with 200  $\mu$ l of 0.1% sodium dodecyl sulphate (SDS) for 1 min. Methanol: 2-propanol (80:20) containing 700  $\mu$ l tocopherol acetate (internal standard) were added to the sample and mixed vigorously for 3 min, inverted shaking for 1.5 h and centrifuged at 3000 rpm for 10 min. Supernatant (2 ml) was then evaporated under nitrogen gas at 45°C. The precipitate was reconstituted with 200  $\mu$ l of freshly prepared mobile phase (acetonitrile:methanol, 3:7); then 25(OH)D<sub>3</sub> and  $\alpha$ -tocopherol levels

were measured by a reverse-phase HPLC system with a dual wavelength UV-VIS detector using HP1100, Agilent system with a quaternary pump. The mobile phase was delivered to a C18 Luna Phenomenex column (150  $\times$  4.60 mm) at a flow rate of 1 ml/min, maintained at 30°C. The UV-VIS detector set at the wavelength of 265 and 292 nm were the  $\lambda$ max of standard 25(OH)D<sub>3</sub> and  $\alpha$ -tocopherol, respectively.

### Ethics

Ethical approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, Chiang Mai University (Protocol No. 31/2551) that the animals used in the study conformed to international and national guideline for ethical conduct on the care and use of animals.

### Statistical analyses

All results were presented as mean  $\pm$  SE. The difference among groups and data correlation were obtained by one-way ANOVA and Pearson correlation, respectively. Statistical significance was considered at  $p < 0.05$ .

## RESULTS

The average body weights of male and female rats consuming Thai brown rice in the control, low dose and high dose groups were not significant among groups. The serum MDA level of the high dose group was lower than those of the low dose and the control groups. The mean values of MDA in males high dose (98.48  $\pm$  5.15 nM) and low dose (109.38  $\pm$  4.28) were significantly lower than that in the control (136.36  $\pm$  8.68 nM) at  $p = 0.002$  and  $p = 0.026$ , respectively. Similar pattern was found in the female rats. The MDA levels in the control, low dose and high dose groups were 137.30  $\pm$  7.69, 89.83  $\pm$  13.13 and 79.98  $\pm$  14.84 nM, respectively. The MDA level in high dose and low dose groups were significantly lower than those in the control at  $p = 0.015$  and  $p = 0.046$ , respectively (Table 1).

Rats that consumed Thai brown rice tended to have high level of FRAP in serum. FRAP in the high dose males (209.28  $\pm$  9.18  $\mu$ M) was significantly higher than that in the control (164.96  $\pm$  15.75  $\mu$ M) at  $p = 0.043$ , while in the females it was not (Table 1).

Serum 25(OH)D<sub>3</sub> levels in the control, low dose and high dose males were 82.26  $\pm$  6.43, 106.67  $\pm$  2.90 and 125.33  $\pm$  9.06  $\mu$ M, respectively. In females, 25(OH)D<sub>3</sub> was high in the control (96.10  $\pm$  4.15  $\mu$ M), low dose (111.43  $\pm$  5.81  $\mu$ M) and high dose (121.22  $\pm$  3.52  $\mu$ M) groups. Serum 25(OH)D<sub>3</sub> of the high dose groups was more significantly different than that of the controls in both males ( $p = 0.001$ ) and females ( $p = 0.005$ ); Additionally, the level of MDA was strongly inversely related with 25(OH)D<sub>3</sub> ( $r = -0.656$ ,  $p < 0.001$ , Figure 1a).

The  $\alpha$ -tocopherol level in male rats, the high dose group

**Table 1.** Serum malondialdehyde (MDA), ferric reducing antioxidant power (FRAP), 25(OH)D<sub>3</sub> and  $\alpha$ -tocopherol levels in rats.

Groups (n = 36)	Mean $\pm$ SE (p value)			
	MDA (nM)	FRAP ( $\mu$ M)	25(OH)D <sub>3</sub> ( $\mu$ M)	$\alpha$ -tocopherol ( $\mu$ M)
<b>Males (18)</b>				
Control (6)	136.36 $\pm$ 8.68 (0.026) <sup>1*</sup>	164.96 $\pm$ 15.75 (0.369) <sup>1</sup>	82.26 $\pm$ 6.43 (0.060) <sup>1</sup>	113.40 $\pm$ 9.54 (1.000) <sup>1</sup>
Low dose (6)	109.38 $\pm$ 4.28 (0.728) <sup>2</sup>	191.09 $\pm$ 7.14 (0.819) <sup>2</sup>	106.67 $\pm$ 2.90 (0.196) <sup>2</sup>	116.60 $\pm$ 16.96 (0.021) <sup>2*</sup>
High dose (6)	98.48 $\pm$ 5.15 (0.002) <sup>3**</sup>	209.28 $\pm$ 9.18 (0.043) <sup>3*</sup>	125.33 $\pm$ 9.06 (0.001) <sup>3**</sup>	177.07 $\pm$ 13.65 (0.015) <sup>3*</sup>
<b>Females (18)</b>				
Control (6)	137.30 $\pm$ 7.69 (0.046) <sup>1*</sup>	156.77 $\pm$ 11.48 (1.000) <sup>1</sup>	96.10 $\pm$ 4.15 (0.097) <sup>1</sup>	137.93 $\pm$ 4.18 (0.253) <sup>1</sup>
Low dose (6)	89.83 $\pm$ 13.13 (1.000) <sup>2</sup>	174.91 $\pm$ 16.45 (1.000) <sup>2</sup>	111.43 $\pm$ 5.81 (0.459) <sup>2</sup>	162.15 $\pm$ 13.48 (1.000) <sup>2</sup>
High dose (6)	79.98 $\pm$ 14.84 (0.015) <sup>3*</sup>	178.55 $\pm$ 19.38 (1.000) <sup>3</sup>	121.22 $\pm$ 3.52 (0.005) <sup>3**</sup>	166.91 $\pm$ 7.60 (0.128) <sup>3</sup>

1: p-value of control and low dose groups, 2: p-value of low dose and high dose groups, 3: p-value of control and high dose groups, \* and \*\*: statistically significant at  $p < 0.05$  and  $< 0.01$ , respectively.

(177.07  $\pm$  13.65  $\mu$ M) was statistically significant than that of the control (113.40  $\pm$  9.54  $\mu$ M) ( $p = 0.015$ ) and low dose (116.60  $\pm$  16.96  $\mu$ M) ( $p = 0.021$ ) groups, but was not significantly different between the control and low dose groups ( $p = 1.000$ ). Although the  $\alpha$ -tocopherol levels between groups of female rats were not significant, the high dose group tended to have higher level than those from the low dose and the control groups (Table 1). Moreover, the mean value of MDA was also inversely related to that of  $\alpha$ -tocopherol ( $r = -0.538$ ,  $p = 0.001$ , Figure 1b).

## DISCUSSION

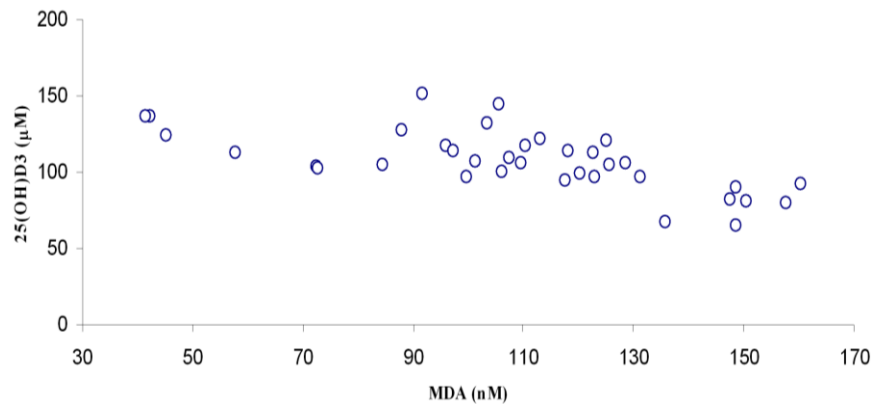
Oxidative stress on lipid, protein and DNA leading to cellular damage is the main cause of clinical diseases (Favier, 2006; Fiers, 1999; Halliwell, 1996). In general, oxidative stress was implicated on cells as a result of an increase in radical generation, a decrease in antioxidant defense and a failure to repair oxidative damage (Hayes and McLellan, 1999).

Brown rice is the main source of antioxidant components (Tian, 2004). Two months after feeding with Thai brown rice, the results of the present study showed that the high dose group tended to have lower serum MDA level than the low dose and control groups. MDA

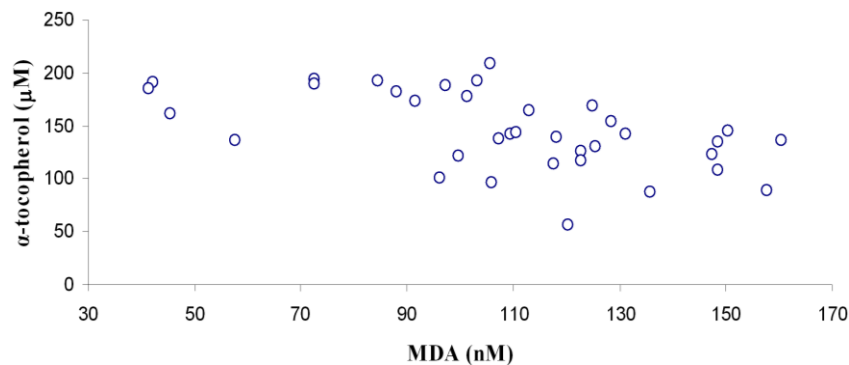
levels of the high dose and low dose groups were significantly lower than that in the controls in both male and female rats (Table 1). The results pinpoint that rats consuming Thai brown rice may have a crucial role in reducing stress marker.  $\alpha$ -Tocopherol's role in oxidative stress prevention had been reported (Halliwell, 1997; Suwannalert et al., 2007). We found that  $\alpha$ -tocopherol level in high dose group tended to have higher level than those in the low dose and the control groups. In addition, the mean value of MDA was inversely related to that of  $\alpha$ -tocopherol ( $r = -0.538$ ,  $p = 0.001$ ) (Figure 1b). These results indicate that Thai brown rice exerts an antioxidant activity.

The FRAP, serum antioxidant, in the high dose group was statistically significant than that in the control ( $p = 0.043$ ) males whereas in the females it was not (Table 1). This result indicate that not only the antioxidant substance play a role in decreasing oxidative stress but also a non radical pathway. This agrees with the finding of Jones (2008) that the major cause of oxidative stress could be generated without free radical. It occurs as a consequence of non radicals that disrupt the function of the thiol redox circuit, especially the glutathione (GSH) system (Hayes and McLellan, 1999; Jones, 2008).

Interestingly, we found that the storage vitamin D [25(OH)D<sub>3</sub>] in the high dose group was higher than the controls in both males ( $p = 0.001$ ) and females ( $p = 0.005$ )



**Figure 1a.** Correlation between serum MDA and 25(OH)D<sub>3</sub> levels.



**Figure 1b.** Correlation between serum MDA and α-tocopherol levels.

(Table 1). Moreover, the mean level of MDA was strongly inversely related to those of 25(OH)D<sub>3</sub> ( $r = -0.656$ ,  $p < 0.001$ ) (Figure 1a). This correlation indicates that storage vitamin D has a crucial role in diminishing MDA. Vitamin D was reported as immunosuppressive lipid peroxide, that stabilize lysosomal membrane and protect nuclear structure (Chen et al., 2003; Kallay, 2002; Wiseman, 1993). Brown rice is a high source of tocopherol, tocotrienols, ferulic acid, oryzanols and phenolic compounds (Tian, 2004). The phenolic compounds were also able to raise the serum level of the active form of vitamin D<sub>3</sub> due to their inhibitory activity on the enzyme degradation of 1,25-dihydroxyvitamin D<sub>3</sub> and its involvement in another enzyme that synthesize 1,25 dihydroxyvitamin D<sub>3</sub> (Wietrzyk, 2007). GSH was reported as a reducing equivalent and a defense system against reactive species (Kehrer and Lund, 1994). The action of GSH has been reported to maintain a cellular reducing state that requires nicotinamide adenine dinucleotide phosphate (NADPH) and G6PD (Bao, 2008; Jones, 2006). G6PD was dose- and time-dependent induced by 1α, 25-dihydroxyvitamin D<sub>3</sub> (Bao, 2008).

## Conclusion

In this study, the rats consuming Thai brown rice possessed a low level of oxidative stress marker and high levels of storage vitamin D and α-tocopherol. Thus, dietary red color strain of Thai brown rice supplementation may have a beneficial role in the prevention of oxidative stress through both the radical and non radical defense mechanisms.

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