Research Article

# Neuroprotective abilities and cognitive enhancing effects of red jasmine rice extract in animal model of Alzheimer's disease

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Naresuan Phayao J. 2015:8(2):67-73.

## Abstract

The aim of the study is to evaluated the neuroprotective effect of the red jasmine rice extract and tested the beneficial effect on memory impairments induced the rat ethylcholine mustard azirinium ion (AF64A) injection model of Alzheimer's disease. After that the male Wistar rats were supplemented with the red jasmine rice extract at doses of 250 and 500 mg/kg BW once daily continually for 2 weeks. Cognition was evaluated using the Morris water maze test. Both doses of extract supplementation significantly prevented changes of spatial memory of AF64A treated rats. The cognitive enhancement of the extract was found to be related to its ability to inhibit the degeneration of neurons in hippocampus. It is suggested that red jasmine rice extract may prevent the dementia of the Alzheimer type.

**Keywords**: Alzheimer's disease, red jasmine rice, neurodegeneration, antioxidant, cognitive function

## Introduction

Nowadays, Alzheimer's disease (AD) is becoming a greater medical and social problem. Only effective treatment for AD targets the cholinergic system using anti-cholinesterase compounds. [1] However, many of the existing drugs have adverse side effects such as bioavailability problems and gastrointestinal disturbance. [2,3] There is therefore a need to develop novel therapeutic approaches to treat this disease.

Oxidative stress is recognized as one of the primary processes underlying the initiation and progression of AD. [4] Interestingly, intake of polyphenols through diets rich in fruits and vegetables was stated to reduce incidence of certain age related neurological disorders including macular degeneration and dementia. [5, 6] Therefore, these data suggest that high dietary or supplemental consumption of antioxidants in people may reduce the risk of AD. Thus, the market of herbal extracts possessing high antioxidant activity and improve memory continues to increase.

Several studies have shown that the red rice is the most nutritious of the rice and also a good source of polyphenolic compound, anthocyanin and proanthocyanidin. [7-9] However, proanthocyanidins were identified as dominant antioxidants in red rice varieties. [10] Recently, *in vitro* and *in vivo* anticancer activities of proanthocyanidin in grape seeds have been

Received: 6 March 2015; Accepted: 17 June 2015

reported with regard to anti-angiogenesis and cancer invasion. [11] In addition, previous studied demonstrated that red rice extract reduced cancer cell invasion via reduced MMP-2,-9 activity and expression. [12] However, the potential of red jasmine rice, as neuroprotective agent, has not been properly investigated. Thus, the present study was undertaken to evaluate the effects of this extract on neuroprotection and cognition, in the rat AF64A injection model of AD.

## Material and Method

### Plant material

Whole grains of red jasmine rice were harvested from Chiangmai province, Thailand. A voucher specimen number was certified by the herbarium at the Flora of Thailand, Faculty of Pharmacy, Chiangmai University (voucher specimen no. 023108) which was kept for future reference.

### Red jasmine rice extraction

One hundred grams of red rice grains from each variety were soaked in solvents, namely water at 25°C, hot water at 50°C and 70% ethanol at 25°C; ratio 1:5 (w/v) in a serial manner. Extraction was done for 40 min using an ultrasonic bath. A constant temperature was set throughout the extraction. The extracts were centrifuged at 5,000 rpm for 20 min and then the supernatants were collected. Each solvent extraction was repeated twice and each of the extract solution was combined and dried under vacuum rotary evaporator. The percent yield obtained was 6.82 %. All dried extracts were kept in the freezer (-20°C) until used.

### Animals

Adult male Wistar rats (180 ± 20 g, 8 weeks old) were obtained from National Animal Center, Salaya, Nakornpathom and they were housed in group of five per cage in standard metal cages at 22 ± 2°C on 12: 12 h light-dark cycle. All animals had free access to standard rodent pellet diet and water *ad libitum*. The food was withdrawn 24 h before the surgical procedure. Experiments were conducted in accordance with the Animal Ethics Committee of the University.

### Experimental procedure

#### AF64A administration

The animals were anesthetized with 400 mg/kg body weight (BW) chloral hydrates intraperitoneally and placed on a stereotaxic frame and skull was exposed. The stereotaxic coordinates for lateral ventricle were measured accurately as anteroposterior 0.8 mm, lateral 1.5 mm and dorsoventral 3.6 mm relative to bregma and ventral from dura with the tooth bar set at 0 mm. Through a skull hole, a 28-gauge Hamilton® syringe of 10 µL attached to a micro-injector unit and piston of the syringe was lowered manually into each lateral ventricle. The lesioned groups received a bilateral intracerebroventricular injection of AF64A (2 nM/2 µL). [13] The sham groups underwent the same surgical procedures, but same volume of artificial cerebrospinal fluid (ACSF) was injected instead of AF64A. After surgery, the rats were housed individually and had access to food and water ad libitum.

#### Experiment I

Experiment I was carried out to evaluate the post-treatment effect of red jasmine rice (250 and 500 mg/kg body weight) extracts on the cognitive function. The rats were divided into four groups of 8 animals each. Group 1) vehicle + ACSF: rats were orally given saline which served as vehicle to suspend the red

jasmine rice extracts fed via feeding needle for a period of 2 weeks after the administration of ACSF; group 2) Vehicle + AF64A: rats were treated as similar as group I except that the administration of AF64A, a cholinotoxin, was performed instead of ACSF administration; group 3) and group 4) red jasmine rice + AF64A: rats were separately treated with the red jasmine rice extracts at doses of 250 and 500 mg/kg BW for 2 weeks after AF64A lesion.

**Experiment II**

Experiment II was conducted to evaluate the neuroprotective effect of red jasmine rice (250 and 500 mg/kg BW) extracts against AF64A induced neurotoxicity. The rats were divided into four groups as in experiment I.

**Cognitive assessment testing**

The cognitive assessment test was started 2 weeks after AF64A-lesioned rats, spatial learning and memory of animals were tested in Morris water maze [14]. It consisted of a circular water tank (132 cm diameter and 60 cm height) that was partially filled with water (25 ± 2˚C). A non-toxic paint was used to render the water opaque. The pool was divided virtually into four equal quadrants, labeled north–south–east–west. An escape platform (10 cm in diameter) was hidden 2 cm below the surface of the water on a fixed location in one of the four quadrants of the pool. The platform remained in the same quadrant during the entire experiment. Before the training started, rats were allowed to swim freely into the pool for 60 sec without platform. They were given four trials (once from each starting position) per session for 5 days, each trial having a ceiling time of 60 sec and a trial interval of approximately 30 sec. After climbing on to the platform, the animal remained there for 30 sec before the commencement of the next trial. If rat failed to reach the escape platform within the maximum allowed time of 60 sec, it was gently placed on the platform and allowed to remain there for the same interval of time. The time to reach the platform (escape of latency) was measured. Twenty-four hours after from the acquisition phase, a probe test was conducted by removing the platform. Rats were allowed to swim freely into the pool for 60 sec. The time spent in the target quadrant, which had previously contained the hidden platform was recorded (retention time). The time spent in the target quadrant indicated the degree of memory consolidation taken place after learning.

**Histological procedure**

Fixation of the brain was carried out by transcardial perfusion with fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.3. The brains were removed after perfusion and stored overnight in a fixative solution that is used for perfusion. Then, they were infiltrated with 30% sucrose solution at approximately 4˚C. The specimens were frozen rapidly and 30 μM thick sections were made using cryostat. They were rinsed in the phosphate buffer and picked up on slides coated with 0.01% of aqueous solution of a high molecular weight poly L-lysine.

**Nissl staining**

The duplicate coronal sections of brains were stained with 0.75% cresyl violet, dehydrated through graded alcohols (70, 95, 100% 2×), placed in xylene and cover-slipped using DPX mountant.

**Morphological analysis**

Morphological analysis: Five coronal sections of each rat in each group were studied quantitatively. Neuronal counts in hippocampus were performed by eye using a 400× magnification with final field 255 $\mu m^2$ according to the following stereotaxic coordinates: anteroposterior 4.8 mm, lateral ± 2.4 to 6 mm, depth 3 to 8 mm. The observer was blind to the treatment at the time of analysis. Viable stained neurons were identified on the basis of a stained soma with at least two visible processes. Counts were made in five adjacent fields and the mean number extrapolated to give total number of neurons per 255 $\mu m^2$. All data are represented as number of neurons per 255 $\mu m^2$.

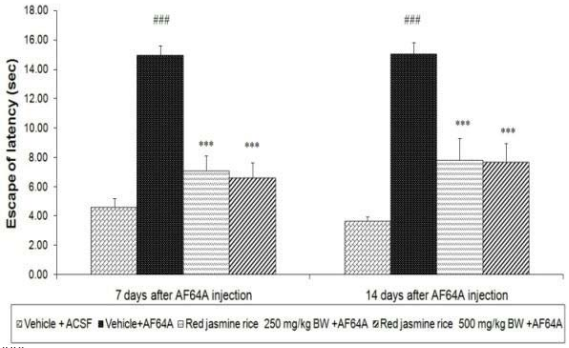
**Statistical analysis**

Data are presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA), followed by Duncan's post hoc test were utilized. A probability level less than 0.05 were accepted as significance.

## Results

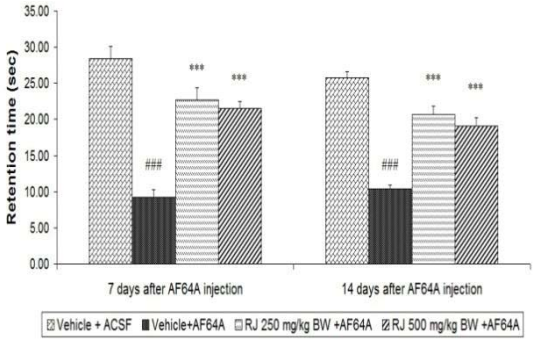
**Cognitive enhancing effect of red jasmine rice extract**

Figure 1 and 2 showed that intracerebroventricular administration of AF64A and vehicle significantly increased escape latency, and decreased retention time ($p<0.001$; compared to vehicle + ACSF) in Morris water maze test. This indicated the memory impairment induced by AF64A. The rats that received AF64A and *both doses* of the red jasmine rice *extract* significantly decreased acquisition, and increased retention time ($p<0.001$; compared to vehicle + AF64A). In addition, the mentioned phenomenon was still observed when the treatment duration was increased to 2 weeks. Therefore, the finding data indicated that red jasmine rice extract improved the memory deficit induced by AF64A.




### p<0.001 compared with vehicle + ACSF treated group,
*** p<0.001 compared with vehicle + AF64A treated group

**Figure 1** The effect of red jasmine rice extract on the escape latency in Morris water maze test. The rats had been orally treated with vehicle or red jasmine rice extract (250 and 500 mg/kg BW) for 2 weeks after AF64A lesioning. Data are presented as mean ± SEM, n = 8 per group

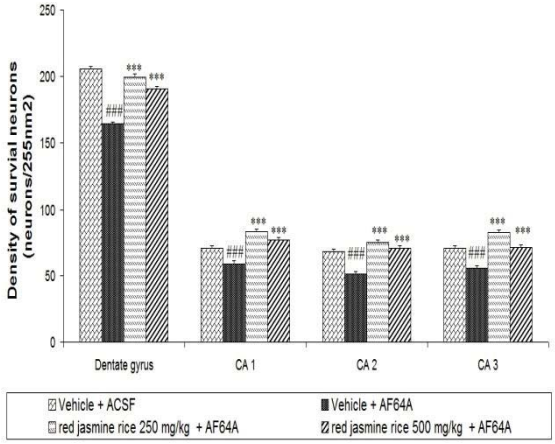



### p<0.001 compared with vehicle + ACSF treated group,
*** p<0.001 compared with vehicle + AF64A treated group

**Figure 2** The effect of red jasmine rice extract on the retention time in Morris water maze test. The rats had been orally treated with vehicle or red jasmine rice extract (250 and 500 mg/kg BW) for 2 weeks after AF64A lesioning. Data are presented as mean ± SEM, n = 8 per group

**Neuroprotective effect of red jasmine rice extract**

Accumulating data demonstrated that learning and memory particularly spatial memory were tightly associated with the function of various brain areas, which in turn depended on the density of survival neurons [15]. Therefore, the study also determined the effect of red jasmine rice extract on the alteration of the survival neurons densities in various subregions of hippocampus, the area which played an important role on learning and memory. As shown in Figure 3, AF64A produced significant reduction in survival neurons densities in all areas of hippocampus ($p<0.001$ all; compared to vehicle + ACSF). In addition, statistical analysis revealed that there was a significant induction in survival neurons densities of both doses of red jasmine rice extract plus AF64A treated group in all areas as mentioned earlier ($p<0.001$ all; compared to vehicle + AF64A).




### p<0.001 compared with vehicle treated group,

*** p<0.001 compared with vehicle + AF64A treated group

**Figure 3** The effect of red jasmine rice extract on the density of survival neurons in AD model. Data are presented as mean ± SEM, n = 8 per group

## Discussion

The major finding of the study is that rats consuming the *both dosages* of the red jasmine rice extract (250 and 500 mg/kg BW) for 2 weeks after AF64A lesion had dramatically induce the densities of survival neurons in hippocampus resulted in the improvement of memory deficit in animal model of AD induced by AF64A. The rats also showed better cognitive function and learning when tested in a water maze.

Recently, a pile of evidence demonstrated that oxidative stress is associated with the pathogenesis of AD, and producing the neuronal damaged mediating the cognitive deficits of the disease. [16,17] During the last few years, antioxidant has received special attention as dietary supplements. Many studies reported that reversals in age-related memory declines might be accomplished by increasing the dietary intake possessing high antioxidant activity. [18,19] Thus, many researchers focused on the beneficial effects of supplement possessing a capability to improve antioxidant activity were considered to be a potential candidate for neuroprotective agent against AD.

Although a close relationship exists between severity of memory deficit and neurodegeneration, it has been suggested that this relationship might be dependent on enhanced oxidative stress. [20] The present study showed a strong correlation between the cognition and the neuroprotective effect of red jasmine rice extract. It should be considered that the densities of survival neurons in all subregions of hippocampus were higher than those obtained from the vehicle + AF64A treated group. Therefore, red jasmine extract possessed the neuroprotective effect.

Previous studies have shown that red rice exhibit higher antioxidant activity and higher

phenolic compounds. [21] However, proanthocyanidins were identified as dominant antioxidants in red rice varieties. This suggested that there were many active ingredients might exert cognitive enhancing effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a cognition and memory process. By the way, the increasing doses of this extract might increase all ingredients concentration and result in the masking effect of active ingredient. It might attribute to the lack of dose-dependent response in the study.

In conclusion, the red jasmine rice extract had demonstrated a potential neuroprotective agent and cognitive enhancer for AD in animal model. However, further researches are still required to detail underlying mechanism.

## Acknowledgements

The study was financially supported by the grants of the National Research Council of Thailand.

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