The Effects of Resveratrol on Oxidative Stress in Healthy Subjects

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Abstract

Oxidative stress describes a condition which cellular antioxidant defenses are inadequate to completely detoxify free radicals. Oxidative stress is an important role in the pathogenesis of several diseases such as cardiovascular disease, cancer, diabetic mellitus and aging. Resveratrol is a polyphenolic compoundand has antioxidative properties. However, the effects of resveratrol on oxidative stress in healthy subject has not been studies. The objective of this study was to compare the effects of resveratrol with placebo on oxidative stress in healthy subjects. 40 Thai healthy subjects were recruited and randomly divided into 2 groups, 20 subjects each. The subjects in the first group were assigned to take resveratrol 250 mg/day for 8 weeks and the second group were assigned to take placebo which contained maltodextrin 250 mg/day for 8 weeks. FORT (free oxygen radical test) and FORD (free oxygen radicals defense) were measured in before and after the study. We found that in the group taking resveratrol , FORT levelsshowed slight decrease when compared to the starting point, but without statistical significance (p=0.29). No change was observed in the group taking placebo. There was no significant difference on the change of FORT before and after study between the two groups (p=0.50). FORD levels showed no significant difference before and after the study in both groups. There was no difference in the FORD change between the two groups (p=0.984).

In healthy subjects, the effects of resveratrol compared to placebo on oxidative stress showed no significant difference. This may be because we were not able to control lifestyle patterns of subjects such as eating behavior, exercise and psychologic stress. Thus we assumed lifestyle patterns were more important factors than taking resveratrol 250 mg/day which affected oxidative stress condition.The study period of 8 weeks may be too long which contributed to unhealthy lifestyle patterns accumulated, affecting oxidative stress condition. FORT and FORD tests are simple, rapid, reliable and the useful tools for physician in mornitoring oxidative stress of the patients who intend to improve their lives. Evaluation of oxidative stress is useful in preventive medicine as a precocious diagnosis pathological diseases caused by oxidative stress.

Keywords: Oxidative stress/Resveratrol

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1. Introduction

Oxidative stress describes a condition which cellular antioxidant defenses (superoxide dismutase, catalase ,glutathione peroxidase, albumin, ceruloplasmin, vitamin C, vitamin E, ß-carotene, reduced glutathione and uric acid) are inadequate to completely detoxify free radicals that have been generate because of excessive production of reactive oxygen species (ROS), loss of antioxidant defenses, or typically both.

Oxidative stress is an important role in the pathogenesis of several diseases such as atherosclerosis, cardiovascular disease, cancer, diabetic mellitus, obesity and aging.(Piconi & Ceriello, 2003)

Resveratrol (trans-3,5,4’-trihydroxystilbene) is a polyphenol phytoalexin present in a variety of plant species, including white hellebore, Polygonum cuspidatum, grapes, peanuts and mulberries.Many studies have shown that resveratrol can prevent or slow the progression of a wide variety of diseases, including cancer, cardiovascular disease, ischaemic injuries, and Alzheimer ’sdisease.The beneficial effects are thought to be due to its antioxidative properties

because it is known as a robust scavenger of superoxide, hydroxyl radicals, and peroxynitrite.However, the effects of resveratrol on oxidative stress in healthy subject has not been studies.

2. Objective

The objective of this study was to compare the effects of resveratrol with placebo on oxidative stress in healthy subjects.

3. Materials and methods

40 Thai healthy subjects were recruited and randomly divided into 2 groups, 20 subjects each. The subjects in the first group were assigned to take resveratrol 250 mg/day for 8 weeks and the second group were assigned to take placebo which contained maltodextrin 250 mg/day for 8 weeks. FORT (free oxygen radical test) and FORD (free oxygen radicals defense) were measured in before and after the study.

Subjects

40 Thai healthy subjects were recruited from Mae Fah Luang hospital in Bangkok and randomly divided into 2 groups, 20 subjects each. The subjects' age were between 20-50 years old.The subjects were included provided that theydid not have any disease and did not take any supplements or medicines. The subjects were excluded if they had any disease such as atherosclerosis, coronary heart disease, pulmonary disease, neurologic disease, psychiatric disease and autoimmune disease, or they were current smokers, pregnancy or lactating.

The FORT test (Free oxygen radical test)

FORT test (Callegari, Parma, Italy)is a colorimetric test based on the ability of transition metals, such as iron, to catalyze the breakdown of hydroperoxide (ROOH) into derivative radicals, according to the Fenton reaction.

When 20 µL of blood sample was dissolved in an acidic buffer ,the hydroperoxides reacted with the transition metal irons liberated from the proteins in the acidic medium and were convert to alkoxy ($RO^{\cdot}$)and peroxy ($ROO^{\cdot}$)radicals. The radical species produced by the reaction interact with an addictive (phenylenediamine derivative ($2CrNH_2$))that forms a colored, fairly long-lived radical cation evaluable by spectrophotometer at 505 nm. The intensity of the color correlates directly to the quantity of radical compounds and to the hydrogenperoxides concentration and consequently to the oxidative status of the sample according to the Lambert-Beer law.

$R\text{-}OOH + Fe^{2+} \longrightarrow RO^{\cdot} + OH^{-} + Fe^{3+}$

$R\text{-}OOH + Fe^{3+} \longrightarrow ROO^{\cdot} + H^{+} + Fe^{2+}$

$RO^{\cdot} + ROO^{\cdot} + 2CrNH_2 \longrightarrow ROO^{-} + RO^{-} + [\,CrNH_2^{+\cdot}\,]$

Results are expressed as FORT U)FORT units(, whereby 1 FORT U corresponds to 0.26mg/L H_2O_2.The test is completed in 6 minutes. Data are available to suggest that the FORT test can satisfactorily assess the level of oxidative radicals in whole blood (Mantovani et al., 2004; Garelnabi et al., 2008; Abramson et al., 2005; Ridker, Brown, Vaughan, Harrison & Mehta, 2004; Harris et al., 2007;Pavlatouet al., 2009)

The FORD test (Free oxygen radicals defense)

The FORD test (Callegari, Parma, Italy)is a colorimetric test. The FORD test uses performed stable and colored radicals and determines the decrease in absorbance that is proportional to the blood antioxidant concentration of the sample according to the Lambert–Beer law.

In the presence of an acidic buffer (pH=5.2)and a suitable oxidant ($FeCl_3$), the chromogen that contains 4-amine-*N*,*N*-diethylaniline sulfate forms a stable and colored radical cation photometrically detectable at 505 nm.
Antioxidant compounds in the sample reduce the radical cation of the chromogen ,quenching the color and producing a decoloration of the solution. Which is proportional to their concentration.The absorbance values obtained for the samples are compared with a standard curve obtained using Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), a permeable cell derivative of vitamin E commonly uesd as an antioxidant.

$$\text{Chromogen}_{(\text{no color})} + Fe^{2+} + H^{+} \longrightarrow \text{Chromogen}^{\cdot+}_{(\text{purple})}$$

$$\text{Chromogen}^{\cdot+}_{(\text{purple})} + AOH \longrightarrow \text{Chromogen}_{(\text{no color})} + AO$$

The assay is completed in 6 minutes.Data is available to suggest that the FORD test can satisfactorily assess the level of oxidative radicals in whole blood (Pavlatouet al., 2009)

Statistical analysis

We report all data as mean ± SD. Compared FORT and FORD levels 's before and after study were examined using the paired t-testorthe Wilcoxon match-pairs signed-rank test.
Compared FORT and FORD levels between the two groups were examined using the t-testorthe Mann- Whitney U test.P value <0.05 (2 –tailed) were considered statistically significant.

Result

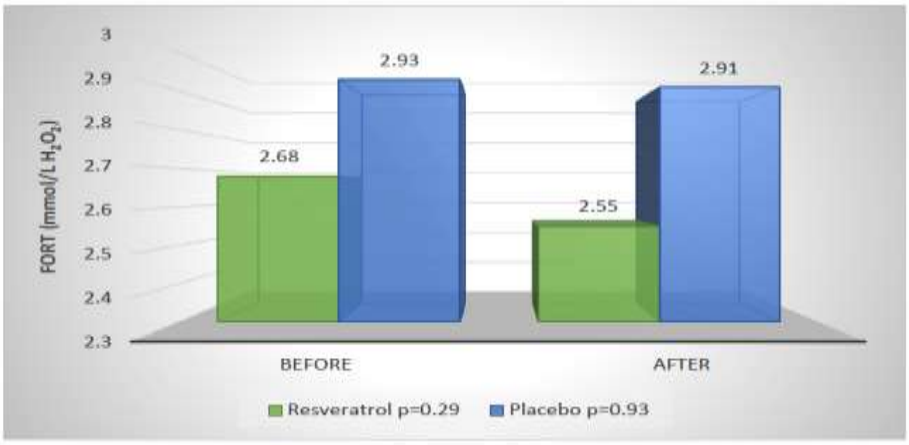
Table 1. General data of subjects

Data of Subjects	N	%	Mean	SD	Median	Min	Max
Gender							
Male	17	42.5					
Female	23	57.5					
Age			30.08	7.42	30	20	49
Occupation							
Official	15	37.5					
Employee	15	37.5					
Student	7	17.5					
Etc.	3	7.5					

Table 2. Compared FORT and FORD levels's before the study of both groups

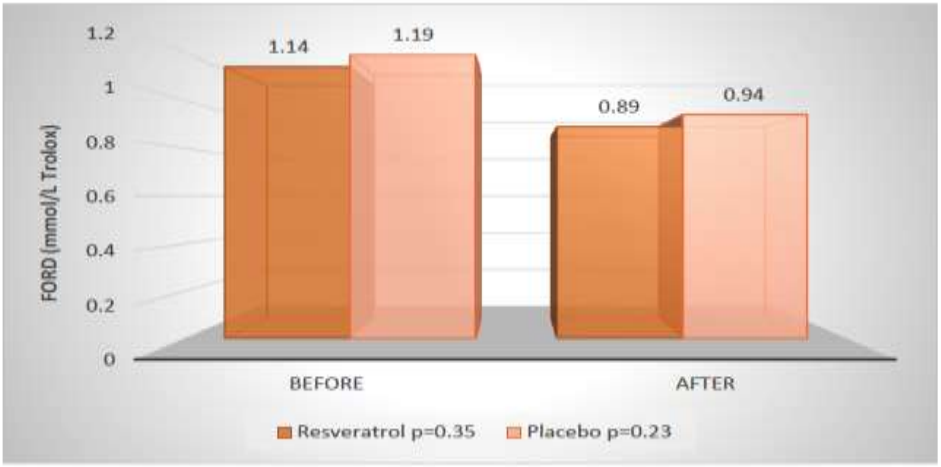
Test	Resveratrol	Placebo	p-value
FORT (mmol/L H_2O_2)	2.68 ± 0.65	2.93 ± 0.84	0.315
FORD (mmol/L Trolox)	1.14 ± 1.11	1.19 ± 0.91	0.436

Compared the FORT levels' before the study between the two groups showed no significant difference (p=0.315) and compared the FORD levels' before the study between the two groups showed no significant difference (p=0.436)
Thus,FORT and FORD levels's before the study did not affect the results of this study. (Table 2.)



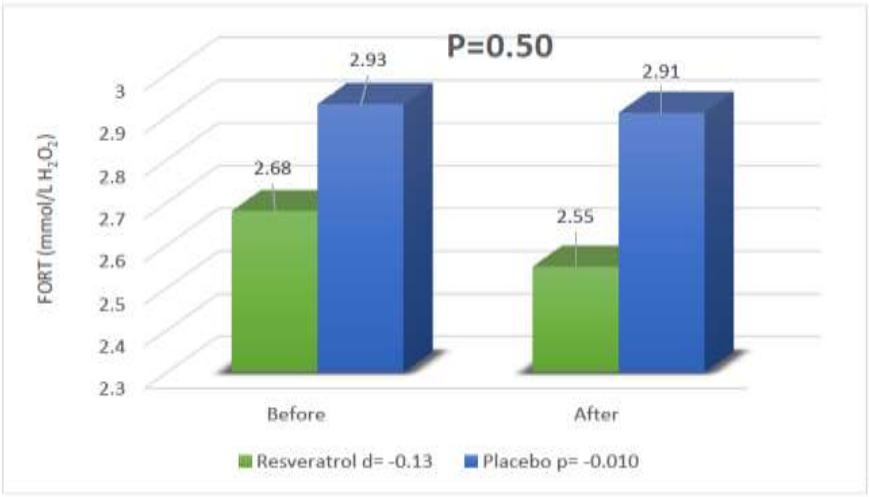
Picture 1. Compared FORT level 's before and after the study of both groups

In the group taking resveratrol , FORT levelsshowed slight decrease when compared to the starting point, but without statistical significance (p=0.29) and no change was observed in the group taking placebo (p = 0.93).(picture1.)



Picture 2. Compared FORD levels's before and after the study of both groups

In the group taking resveratrol , FORD levels which compared before and after the study showed no significant difference in levels (p=0.35) and in the group taking placebo , FORD levels which compared before and after the study showed no significant difference in levels (p=0.23). Thus, FORD levels showed no significant difference before and after the study in both groups. (picture2)



Picture 3. Compared the changes of FORT levelsof both groups

There was no significant difference on the change of FORT between the two groups (p=0.50). (picture 3)

Picture 4. Compared the changes of FORD levelsof both groups
There was no significant difference on the change of FORDbetween the two groups (p=0.984). (Picture 4)

5. Discussion

In the group taking resveratrol , FORT and FORD levelswhich compared before and after the study showed unstatistical significance (p=0.29), (p=0.35) and the results were same in group taking placebo.There were no dfference in the FORT and FORD change between the two groups (p=0.50), (p=0.984)

The results of this study **disagree** with the study of Munehiro, Shinji, Noriko & Daisuke (2011) and the study of Cho & Kim (2009) that because their studies did in vitro and in animals so it was easy to control other factors such as food, exercise and environment.

The results of this study **agree** with the study of Lesgards, et al. (2002) and the study of Moller, et al. (1996) that the antioxidant potential was negatively related to tobacco smoking, psychologic stress, alcohol consumption, moderate vetgetable , low fruit, low fish consumption and high natural ultraviolet light exposure. Lifestyle patterns that truly contributed to the variation of individual antioxidant capacities.

We found the effect of resveratrol compared to placebo on oxidative stress in healthy subjects showed no significant difference. This may be because we were not able to control lifestyle patterns of subjects such as eating behavior, exercise and psychologic stress. Thus, we assumed lifestyle patterns were more important factors than taking resveratrol 250 mg/day which affected oxidative stress condition.
The study period of 8 weeks may be too long which contributed to unhealthy lifestyle patterns accumulated, affecting oxidative stress condition.

6. Conclusion

In healthy subjects, the effects of resveratrol compared to placebo on oxidative stress showed no significant difference. Lifestyle patterns were more important factors than taking resveratrol 250 mg/day which affected oxidative stress condition.
FORT and FORD tests are simple, rapid, reliable and the useful tools for physician in mornitoring oxidative stress of the patients who intend to improve their lives.Evaluation of oxidative stress is useful in preventive medicine as a precocious diagnosis pathological diseases caused by oxidative stress.
In the further studies should evaluate many doses of resveratrol for the optimal effects on oxidative stress and also should control the lifestyle patterns of subjects such as eating behavior, exercise and psychologic stress , that for control factors can affect the oxidative stress.

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