Effects of Syzygium gratum on antioxidant system in β-thalassemia/Hb E patients
ผลของผักเม็กต่อระบบต้านออกซิเดชันในผู้ป่วยธาลัสซีเมีย ฮีโมโกลบินอี

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ABSTRACT

Thalassemia is one of the genetic disorders, resulting from an abnormal hemoglobin synthesis. The red blood cells containing abnormal hemoglobin are destroyed and some of these thalassemia patients need regular blood transfusion that results in an increased accumulation of iron in the body. The presence of systemic iron overload will catalyze the generation of reactive oxygen species and leads to the oxidative stress and tissue injuries. Supplementation with antioxidant substances may be beneficial in attenuating the oxidative stress and minimizing organ dysfunction.

The purpose of this work was to evaluate a feasibility study of the use of *Syzygium gratum* (SG) in β-thalassemia/HbE patients and adverse effects. Eleven blood transfusion-dependent thalassemia patients received SG water extract at dose of 0.1 g/kg body weigh/day, but total dose not more than 5 g, in divided twice daily dose for 7 days. Subjects were requested for recording the questionnaire regarding adverse events, and palatability. Blood samples were collected from subjects before and after the study. Blood samples were analyzed for complete blood count, liver function and kidney function tests, lipid peroxidation (MDA), glutathione (GSH), γ-glutamylcysteine ligase (GCL) activity, total antioxidant capacity by ferric reducing antioxidant power (FRAP) assay and vascular reactivity by forearm blood flow measurement.

There was no report of any major side effects, no changes in CBC examination, liver and kidney function tests. GSH levels, GCL activity and vascular reactivity were unchanged upon the consumption of the SG extract. The total antioxidant capacity in the plasma as assessed by FRAP assay was 1032.8 ± 61.7 μEq and after supplementation, it was significantly increased to 1218.9 ± 77.8 μEq (P-value < 0.001). It was concluded that short term intake of SG extract
enhances plasma antioxidant capacity and merits further study.

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บทคัดย่อ

โรคธาลัสซีเมียเป็นโรคทางพันธุกรรม ที่มีความผิดปกติในการสังเคราะห์ฮีโมโกลบิน ทำให้เกิดภาวะเม็ดเลือดแดงแตกผู้ป่วยเหล่านี้บางรายจำเป็นต้องได้รับการถ่ายเลือดซึ่งเป็นผลตามมาทำให้ผู้ป่วยมีภาวะเหล็กเกิน ทำให้มีการสร้างอนุมูลอิสระของออกซิเจน นำไปสู่การเกิดภาวะเครียดออกซิเดชันและการบาดเจ็บของเนื้อเยื่อ การได้รับสารต้านออกซิเดชันอาจจะลดภาวะเครียดออกซิเดชันและลดความผิดปกติการทำงานของอวัยวะที่เกิดขึ้นได้

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาความเป็นไปได้ของการดื่มน้ำต้มผักเม็กในผู้ป่วยธาลัสซีเมียฮีโมโกลบินอีและประเมินผลข้างเคียงที่เกิดขึ้น ผู้ป่วยธาลัสซีเมียจำนวน 11 คนดื่มน้ำต้มผักเม็ก ในขนาดที่ให้ชงดื่ม 0.1 กรัมต่อกิโลกรัมน้ำหนักตัวต่อวัน แต่ไม่เกินวันละ 5 กรัม แบ่งชงดื่มวันละ 2 ครั้ง เป็นเวลา 7 วัน อาสาสมัครตอบแบบสอบถามเกี่ยวกับอาการข้างเคียงและความยอมรับการดื่มน้ำต้มผักเม็ก รับการเก็บตัวอย่างเลือดก่อนและหลังการศึกษาเพื่อประเมิน complete blood count, liver function and kidney function test, lipid peroxidation (MDA), glutathione (GSH), การทำงานของเอนไซม์ γ-glutamylcysteine ligase (GCL), total antioxidant capacity โดยการวัด ferric reducing antioxidant power (FRAP) assay และ vascular reactivity โดยการวัด forearm blood flow

ผลการศึกษาพบว่าผู้ป่วยธาลัสซีเมีย ไม่มีอาการข้างเคียงใด ๆ รุนแรงเกิดขึ้น และไม่มีความผิดปกติจากผลการตรวจทางห้องปฏิบัติการทั้งการตรวจ CBC, liver และ kidney function tests ส่วนระดับของ GSH, การทำงานของเอนไซม์ GCL และการทำงานของหลอดเลือดไม่มีการเปลี่ยนแปลงหลังจากบริโภคน้ำต้มผักเม็ก ระดับของ total antioxidant capacity ในพลาสมาซึ่งประเมินโดยวิธี FRAP มีค่าเท่ากับ 1032.8 ± 61.7 μEq และหลังจากดื่มน้ำต้มผักเม็ก มีค่าเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเป็น 1218.9 ± 77.8 μEq (p-value < 0.001) การศึกษาสรุปว่าการดื่มน้ำต้มผักเม็กระยะสั้นนี้มีผลเพิ่ม antioxidant ในพลาสมาและงานนี้มีคุณค่าที่จะทำการศึกษาต่อไป

Key Words : Thalassemia, *Syzygium gratum*, Antioxidant
คำสำคัญ : โรคธาลัสซีเมีย ผักเม็ก สารต้านอนุมูลอิสระ

Introduction

Thalassemia is one of the genetic disorders, resulting from an abnormal hemoglobin synthesis. (Comporti et al., 2002). It has been reported that the thalassemic red blood cells increased susceptibility to oxidative stress resulting in mild to severe hemolysis in patients (Ciccoli et al., 1999). One of the prominent manifestation is that the red blood cells containing abnormal hemoglobin are destroyed, resulting in the release of free iron or heme from hemoglobin into circulation (Comporti et al., 2002). Some of these thalassemia patients need regular blood transfusion that results in an increased accumulation of iron in the body (Stenner, 2005). The most important measure is to reduce an iron overload state. The presence of systemic iron overload will catalyze the generation of reactive oxygen species and leads to the oxidative stress and ensuring oxidative damage (Shinar and Rachmilewitz, 1990). This is achieved by administration of iron chelator, this method will reduce iron accumulation in tissues and reduce the

Fenton's chemistry. Another adjunct therapy is to enhance body antioxidant system. One possible practice is by an administration of exogenous antioxidant substances. Although the administration of iron chelator is critically important especially for patients who regularly receive blood transfusion, a large number of patients still present the signs of iron overload and organ abnormalities (Gulati et al., 2000). This is due probably to either insufficient doses or noncompliance with deferoxamine (the iron chelator) or the limitation efficacy of the drug (Cunningham et al., 2004; De Sanctis et al., 2006). These suggests that adjuct treatment by the supplement with antioxidant substances to attenuate the oxidative stress may be beneficial (Tesoriere et al., 2001; Unchern et al., 2003). Kwuangthip et al. (2002) demonstrated that some of Thai local vegetables possess strong antioxidant activities in *in vitro* models. *Syzygium gratum* (SG) is one of the favorite local vegetables among Thai people in the Northeast region. It is demonstrated that SG extract can directly scavenge free radicals, inhibit free radical production and has strong reducing power. In addition, it has an antioxidant effect to alleviate vascular dysfunction and reverse the oxidative stress state (Luangaram S et al., 2004). SG was selected on the basis that it has antioxidant activity and it is frequently consumed in daily diet. It is, therefore, conceivable that the plant should be safe and any toxicity should be already recognized. Therefore, the aim of this study was a feasibility study of the use of SG in thalassemia/ Hb E patients and to determine the antioxidant activity in patients.

Materials and Methods

1. Materials

Glutathione, 5,5 dithio-bis-2-nitrobenzoic acid (DTNB), ethylene tetraacetic acid (EDTA), 2,3 naphthalenedicarboxyaldehyde (NDA), ATP, 5-sulfosalicylic acid (SSA), L-glutamic acid, L-cysteine, glutathione reductase (type IV, Baker's yeast), thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), butylated hydroxyluene (BHT), 1,1,3,3-tetraethoxypropane (TEP) were obtained from Sigma (St. Louis, Mo, USA.). NADPH from Aspergillus species was obtained from Boehringer Mannheim GmbH. (Germany). Sodium dihydrogen phosphate (Na_2HPO_4) was obtained from Carlo Erba, Italy. Disodium hydrogen phosphate (Na_2HPO_4), meta-phosphoric acid (MPA), 1-methyl-2 vinyl-pyridium trifate (M2VP), trichloroacetic acid (TCA) were obtained from Fluka (Buch, Switzerland).

2. Plant preparation

The young leaves of *Syzygium gratum* (SG) were boiled with purified water for 30 min, then the water extract was filtered through the cotton wool. Thereafter, the clear water extract was dried by using spray dried technique.The dried powder of SG was kept in an air-tighted container at -20°C.

3. Participants and study protocol

Participants were thalassemia patients. All subjects were given an explanation regarding the proposed study and written informed consent was obtained from all parents prior to the study. Thalassemia subjects were recruited from the Out-Patient Department Unit of Srinagarind Hospital, Faculty of Medicine, Khon Kaen

University. The study protocol was approved by the Khon Kaen University Ethics Committee for Human Research.

Eleven subjects recruited in the study. They were asked to drink a reconstituted powder of SG water extract with clean drinking water at dose of 0.1 g/kg.body weight/day, but total dose not more than 5 g/d in divided twice daily dose for 7 days. Subjects were requested for recording the questionnaire regarding adverse events and palatability. Blood samples were collected from subjects before and after the study.

Blood samples were analyzed for complete blood count, liver function and kidney function test, lipid peroxidation (MDA) level (Draper et al., 1993), glutathione (GSH) level (Tietze, 1969), γ-glutamyl cysteine ligase (GCL) activity (White et al., 2003) and total antioxidant capacity by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996).

Vascular endothelial function was assessed by the forearm blood flow technique (Aggeli et al., 2005; Higashi et al., 2001) with some modifications. Briefly, subjects were fast overnight and rested in the supine position in a quiet and air-conditioned room for 15 min. A mercury-filled-silastic tube (D.E. Hokanson, Inc., Issaquah, Washington, USA) was attached to the widest part of the forearm and an occlusion cuff was placed just proximal to the elbow. The blood flow was measured following by inflating the upper arm-occlusion cuff to 40 mmHg for 10 sec in each of 20 sec/cycle to occlude venous outflow from the arm. The FBF output signal was recorded and analyzed by a Biopac data acquisition system (Biopac System, CA, USA). The reactive hyperemia was induced with a brief ischemia by inflating the upper arm cuff to the pressure of 200 mmHg for 4 min. We used the protocol of 4 min occlusion time (instead of 5 min. usually found in literatures) in order to minimize discomfort to our subjects who are small children and the preliminary study showed that with this protocol, FBF and forearm vasodilatory response to reactive hyperemia are comparable to a previous report (Aggeli et al., 2005). Basal FBF was estimated as the mean value of 10 subsequent measurements. The FBF following reactive hyperemia was measured twice after 10 min rest. Forearm vasodilatory response to reactive hyperemia was expressed as the percentage change of forearm blood flow from baseline to the maximum flow during reactive hyperemia, following the release of occlusion cuff.

Data were present as mean ± SE. and analyzed by paired t-test or Wilcoxson Signed Ranks test, as appropriate. The p-value less than 0.05 was considered statistical significance.

Results

Eleven thalassemia patients were 6 males and 5 females. There was no report of any major side effects during the study period. There were no changes in CBC examination, liver and kidney function tests after the intake of the extract compared to prior study period. Reduced GSH levels were unchanged upon the consumption of the SG water extract. Activity of GCL, a key enzyme required for glutathione synthesis, and vascular reactivity were also unchanged (Table 1). However, there was an increase in plasma MDA levels.

Table 1 Effects of SG water extracts on the level of reduced glutathione, GCL activity and vascular reactivity in thalassemia patients

PARAMETERS	BEFORE	AFTER	P-value
Blood GSH (μM)	323.3 ± 33.9	347.8 ± 46.9	0.541
GCL activity (pmol/mg.prot/min)	153.7 ± 25.8	166.3 ± 25.4	0.361
MDA (μM)	1.97 ± 0.14	2.65 ± 0.19	< 0.001
Reactive hyperemia (%increase)	100.3 ± 7.3	99.5 ± 8.6	0.453

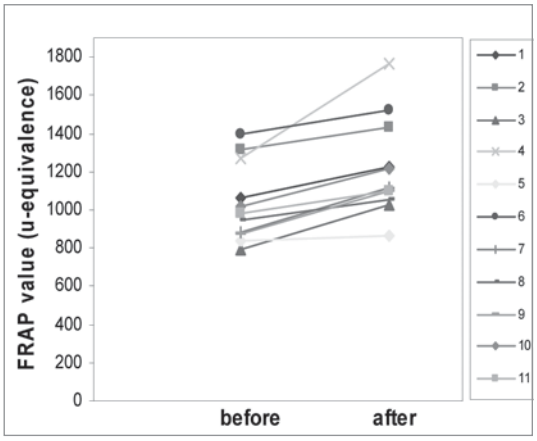

Figure 1 Levels of ferric reducing antioxidant power (FRAP) in plasma samples from thalassemia patients before and after intake of SG extract for 7 days.

The total antioxidant capacity in the plasma as assessed by FRAP assay in patients was consistently increased in all patients (Figure 1). FRAP value in control period was 1032.8 ± 61.7 μEq and after supplementation, it was significantly increased to 1218.9 ± 77.8 μEq, p-value < 0.001.

Discussion

Short term intake of SG water extract was shown to be safe and could increase plasma total antioxidant capacity, even though other antioxidant and oxidant parameters were unchanged. Water extract of SG was shown to reduce the oxidative stress and improve vascular reactivity in phenylhydrazine-induced severe oxidative stress and hemolytic anemia in rats (Luangaram S et al., 2004). The dose employed in that study was relatively much higher compared to our study (1 g/kg body weight/day and 0.1 g/kg/day). For the safety reason and high astringent taste of the extract limit a high dose use in this preliminary study in human. Moreover, thalassemia is a chronic disease, whereas red cell abnormality is genetically inherited (Weatherall, 2000). Since red cell destruction is primarily taken place in bone marrow, most drugs treatment including iron chelation and antioxidant agents could not prevent it. All GSH in the blood is virtually in the red cells. It is, therefore, intake of SG extract is conceivably not effective in increasing blood GSH or number of red cells. On the other hand, there was an increase in lipid peroxidation. This is implied that intake of SG extract may pose some oxidative stress to the body. In our preliminary study in healthy subjects, we did not observe any changes in MDA levels. It is remained to investigate whether the longer term intake of the SG extract produces any undesirable oxidative stress or confer antioxidant effects to the patients. It is well recognized that some agents

enhance antioxidant gene responses by eliciting small oxidative stress such as polyphenol compounds to obtain an antioxidant gene response such as enhanced heme oxygenase-1 expression (Li et al., 2006).

Although there is no report over the active constituents in *Syzygium gratum*, plants in the similar genus such as S. glomeratum and S.mauritianum contain high amount of phenolic compounds and proanthocyanidins (Neergheen et al., 2006). Such compounds are known to possess antioxidant activity. Further work is needed to identify active components in this plant and also other biological activity which provide health benefit. It is concluded that intake SG extract is probably safe and enhanced plasma antioxidant capacity. This merits for further investigation as a food supplement for prevention of oxidative stress related diseases.

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