Reducing Power, Iron Chelating Property and Free Radical Scavenging Activity of *Ventilago denticulata* Willd Leaves Extract in Iron-Loaded HepG2 Cells

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Background: *Iron overload is commonly found in blood transfusion-dependent β-thalassemia patients. Redox excessive iron can catalyze the generation of free radicals, leading to damage of biomolecules and malfunctions of many organs. Therefore, chelation of excessive iron could be an effective therapeutic approach in pathological states associated with oxidative stress.*
Objective: *This study aimed to investigate reducing power, iron chelating property and free radical scavenging activity of Rhangdang leaves extract in iron-loaded HepG2 cells.*
Material and Method: *Rhangdang (Ventilago denticulata willd) leaves were extracted with hydroethanol. The extract was then determined reducing power, free radical scavenging activity, and iron binding/chelating activity.*
Results: *The ethanol extract of Rhang Dang leaves had high reducing power and iron binding ability, and contained considerable free radical scavenging activity. The extract also showed protective activity against H_2O_2 induced oxidative stress in a dose-dependent manner. Interestingly, it decreased the level of labile iron pool in iron loaded HepG2 cells.*
Conclusion: *Rhang Dang (V. denticulata willd) leaves extract contains important active ingredient that possess anti-oxidative and iron-chelating activity. Potentially, the herbal plant would be used for amelioration of iron overload-induced oxidative cell and tissue damage.*

Keyword: *Reducing power, Antioxidant, Reactive oxygen species, Iron chelating, Labile iron pools*

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The high levels of free radicals in living systems are able to oxidize biomolecules, leading to tissue damage, cell death, or various diseases such as neural disorders, cancer, cardiovascular diseases, arteriosclerosis and inflammations[1,2]. Antioxidant compounds can deactivate and scavenge the free radicals by donating hydrogen atom or chelating metals[3,4]. Iron as an essential element participates in many biochemical process in human body due to its vital biochemical activities, including oxygen transport, energy production and cellular proliferation[5]. Iron overload caused by increased iron absorption and multiple transfusions is commonly associated with oxidative stress in -thalassemia major patients. Excess free iron as ferrous iron (Fe^{2+}) is toxic and participates in Haber-Weiss and Fenton reactions to catalyze the conversion of superoxide to hydrogen peroxide and the hydrogen peroxide to highly reactive hydroxyl radicals, respectively. The harmful reactive oxygen species (ROS) can damage DNA, proteins and lipids of a variety of cells and tissues including heart, liver, pancreas, erythrocytes and endocrine glands resulting in organ dysfunction[6]. Non-transferrin bound iron (NTBI), and labile plasma iron (LPI) are toxic forms of the iron that appear in plasma when the transferrin saturation increases. Changes in the labile iron pool (LIP) can be considered a cytosolic equivalent of plasma NTBI influence on intracellular ferritin (Ft) levels[7]. Thus, elevated levels of the LIP lead to an increased accumulation of Ft iron and in extreme cases to the formation of hemosiderin[8]. Essentially, effectiveness and adverse effects of used iron chelators are the important reasons for the development of alternative therapeutic strategies that are safe and effective orally. Natural or phytochemical products that

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exhibit both antioxidant and iron-chelating properties (simply called "bifunctional or two-in-one") are interesting candidates[9].

Rhang Dang (*Ventilago denticulata* Willd) is found extensively as a tropical evergreen in Thailand. The plant is rich in many pharmacological active ingredients[10]. The ethanolic extract of plant also shows anti-inflammation and anti-microbial activity[11]. Rang Dang leaves is often used as a tea products. Frequently drunk, it can help to reduce cholesterol, blood sugar, blood pressure and serve as a relaxant. Our previous study found that the ethanolic extract of Rhang Dang leaves exhibited strong antioxidant activity and prevented hemolysis[14]. Based on these observations, the present study was performed to assess reducing power and iron chelating activity of Rhang Dang leaves extract. Furthermore, we investigated the prevention of iron overload and oxidative stress in the iron-loaded hepatocytes by the Rhang dang leaves extracts.

Material and Method

Chemicals

A stock of ferric nitrate (AAS iron reagent, 1,000 pm, in 0.5% HNO_3: APS Finechem, Seven Hills, Australia) was used as the iron source for other preparations. Stock ferric nitrilotriacetate solution was prepared by consecutive mixing of ferric nitrate with the nitrilotriacetic acid (at a 1:5 molar ratio of Fe^{3+} to chelator). Various iron concentrations were freshly prepared in 10 mM MOPS buffer, pH 7.0, before use, hydrogen peroxide, ascorbic acid, 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA)(sigma-Aldrich, St. Louis, MO, USA) other chemicals and reagents used were of Analytical grade. Rhang Dang leaves (*Ventilago denticulate* Willd) were collected in Chiang Rai Province, Thailand.

Preparation of plant extract

The dried powder of Rang Dang leaves (100 g) were mixed with 1,000 ml of distilled water orethanol (80%v/v) at room temperature for 24 h. The supernatant was collected by filtration through filter paper No. 1. The supernatant was collected and concentrated by using rotary evaporator further lyophilized. The ethanolic and aqueous extract yields were obtained from (46.29%, 32.36%, respectively).

Ferric reducing antioxidant power (FRAP) assay

The reducing power of the Rhang dang leaves extract was determined according to Benzie and Strain with some modifications[12]. The working solution was prepared with acetate buffer (300 mM, pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) and 20 mM ferric chloride ($FeCl_3$ or Fe^{3+}). The extract was mixed with a working solution for 10 minutes at 37°C and absorbance was measured at 593 nm by using a UV-spectrophotometer. Samples were measured in three replicates. Standard curve of ascorbic acid was prepared using the similar procedure. The results were expressed as µmol ascorbic acid/g extract sample. Increases in the absorbance of the samples with concentrations indicated high reducing potentials of the samples.

Iron-binding assay

To determine the iron-binding activity, the ethanolic and aqueous extract from Rhang dang leaves (a final concentration of 1 mg/ml) was incubated with ferric nitrilotriacetate (Fe^{3+}-NTA) (0-200 µM at final concentrations), pH 7.0 at room temperature for 30 minutes. The absorbance of the resulting complexes was measured in the wave length range of 200-800 nm by the use of a scanning double-beam UV-VIS Spectrophotometer (Shimudsu, Japan). Equivalent concentrations of the ethanolic extract from the Rhang dang solution were used as the blank. A stock solution of the Rhang dang leaves extract was freshly prepared by dissolving the extract in 50 mM 3-[N-morpholino] propane sulfonic acid (MOPS) (Sigma-Aldrich Co., St. Louis, MO, USA), pH 7.0 solution. A working Fe^{3+}-NTA solution was freshly prepared by mixing stock ferric nitrate solution (AAS iron reagent, 1,000 ppm in 0.5% HNO3; APS Finechem, Seven Hills, Australia) with anitrilotriacetate (NTA) solution (a molar ratio of Fe^{3+}: NTA = 1: 5).

HepG2 cell culture

HepG2 cells were maintained in DMEM medium (GibcoTM, Life Technologies, USA) supplemented with 10% (v/v) fetal bovine serum (GibcoTM, Life Technologies, USA), 100 U/ml penicillin and 100 U/ml streptomycin, and incubated at 37°C undera normal humidified atmosphere (95% air and 5% CO_2).

Chelation of intracellular LIP in iron loaded hepatocytes

Firstly, HepG2 cells ($5x10^3$ cells/well) were incubated with 0.5 mM ferric ammonium citrate (FAC) solution at 37°C for 24 hour. Secondly, the cells were treated with the Rhang dang leaves extract solutions (0-100 µM) for 24 hour. Thirdly, the treated cells were

incubated with 1 μM calcein-AM solution previously prepared in DMEM at 37°C for 15 minute. Finally, fluorescent intensity (FI) was measured with a 96-well spectrofluoro meter ($\lambda_{excitation}$ 485 nm, $\lambda_{emission}$ 535 nm). Amount of LIP was inversely proportional to the measured FI signal.

Measurement of intracellular reactive oxygen species

DCFH-DA can penetrate into the cells and behydrolyzed by esterase in viable cells to produce 2', 7'-dichlorofluorescein (DCFH) (reduced form), which produced DCFH will be subsequently oxidized by existing ROS to 2', 7'-dichlorofluorescein (DCF) (oxidized form). Increasing of a green fluorescent signal indicates increased intracellular oxidative stress. HepG2 cells ($5x10^3$ cells/well) were incubated with Rhang dang leaves extract at 37°C for 24 hour. The treated cells were was hed three times with the DMEM medium, incubated with DCFH-DA solution (10 μM) for 30 minute, and challenged with H_2O_2 solution (125 μM) for 15 minute. FI was measured with a 96-well spectrofluoro meter ($\lambda_{excitation}$ 485 nm, $\lambda_{emission}$ 535 nm).

Statistical analysis

The results were expressed as mean ± SD.

Results

Reducing power activity

The FRAP assay treats the antioxidants contained in the samples as reductants in a redox-linked colorimetric assay, and the value reflects the reducing power of the antioxidants. This method has been frequently used for a rapid evaluation of the total antioxidant capacity of various antioxidants from grains and vegetables. The ferric reducing antioxidant power of ethanolic and aqueous of Rhang dang leaves extracts is shown in Fig. 1. The FRAP value value for ethanolic extract was higher than that for aqueous extract. The reducing properties of the ethanolic and aqueous extract were 12.5±3.56 and 9.3±5.62 mg ascorbic acid per g extract, respectively. These values suggested that the ethanol extracts of Rhang dang leave had a higher ferric reducing antioxidant power.

Iron binding activity

To determine the chemical binding of iron to Rhang dang leave extract was detect by measuring the optical density of colored complex at the wave length range of 200-800 nm. The result in Fig. 2, 3 showed that the ethanolic and aqueous extract of Rhang dang leave

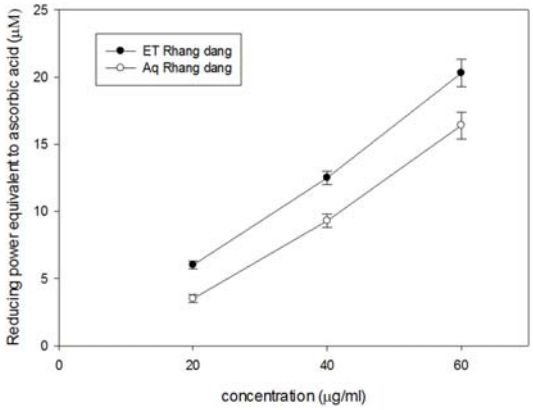

Fig. 1 Reducing activity of the ethanolic (ET) and aqueous (Aq) extract of Rhang dang leaves equivalent to the concentrations of ascorbic acid. Data were obtained from triplicate result of three dependent experiments and shown as mean ± standard deviation (SD).

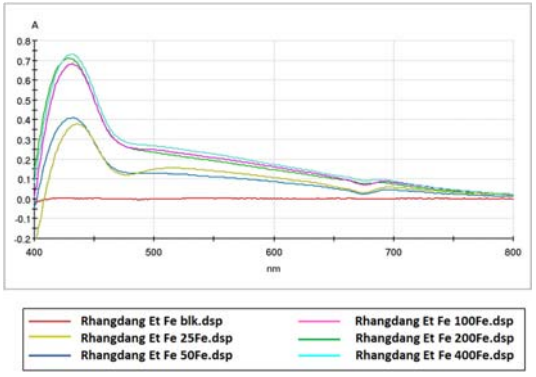

Fig. 2 Spectral analysis of the iron-chelate complex resulting from Fe-NTA (0-400 μM) and ethanolic extract of rhang dang leaves (1 mg/ml).

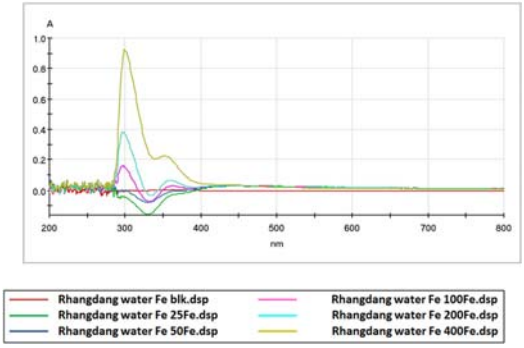

Fig. 3 Spectral analysis of the iron-chelate complex resulting from Fe-NTA (0-400 μM) and aqueous extract of rhang dang leaves (1 mg/ml).

were able to blind the ferric-NTA in a concentration dependent manner. The spectrums of reactivity of ethanolic extract from Rhang dang leave exhibited maximal absorption at 478 nm (Fig. 2) and complex resulting from the reaction of ferric ion and the aqueous extract of Rhang dang leave constituents exhibited a predominant absorption peak at 300 and 353 nm (Fig. 3).

Chelation of LIP in Hepatocyte cell by Rhang dang leaves

The Labile iron pool (LIP) content of HepG2 cell pretreated with FAC for 24 hours was dose dependently decreased in the presence of ethanolic or aqueous extract of Rhang dang leaves (Fig. 4). This decrease in LIP levels was monitored by increase in the FI of the intracellular calcein. The treatment with ethanolicoraqueous extract of Rhang dang leaves tended to lower level of LIP in the FAC-loaded HepG2 cells in a concentration-dependent manner.

Oxidative stress induced by H_2O_2 in HepG2 cells was reduced by Rhang dang leaves extracts

Ability of the Rhang dang leaves extract in scavenging ROS (such as superoxide, hydrogen peroxide and hydroxyl radicals) was investigated in HepG2 cells by using a sensitive and selective DCF fluorescent technique. As shown in Fig. 5, incubation with 400 mM H_2O_2 increased the ROS level in HepG2 cells. After pretreatment with various concentrations of ethanolicor aqueous extract of Rhang dang leave for 24 h, the oxidant burden of HepG2 cells rapidly decreased in a dose-dependent manner.

Discussion

Recent evidences in biomedical sciences emphasis the involvement of free radicals in many diseases, such as brain dysfunction, cancer, heart diseases and immune system. Dietary antioxidant intake may be an important strategy for inhibiting or delaying the oxidation of susceptible cellular substrates, and is thus relevant to disease prevention in many paradigms[13]. Many synthetic drugs protect against oxidative damage but they have many adverse side effects. And alternative solution to the problem is to consume natural antioxidant from food supplements and traditional medicines. Polyphenols compounds in the natural products act as antioxidants by neutralizing ROS and by chelating metal ion. Rhang dang (*Ventilago denticulata* Willd.) belongs to the family Rhamnaceae which is used as medicinal herb in traditional medicine.

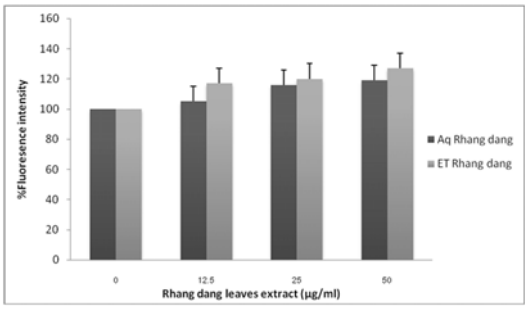

Fig. 4 Dose-response effect of ethanolic and aqueous Rhang dang leaves extract on level of LIP in iron-loaded HepG2 cells. Data obtained from three independent triplicate experiments are shown as Mean ± SD.

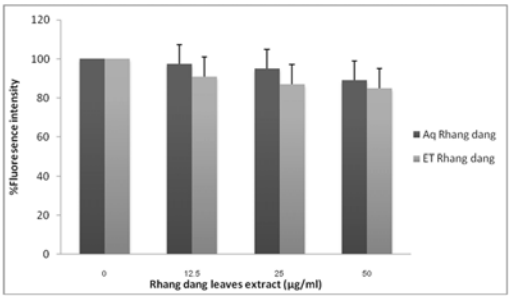

Fig. 5 Dose-response effect of ethanolic and aqueous Rhang dang leaves extract on level of ROS in HepG2 cells. Data obtained from three independent triplicate experiments are shown as Mean ± SD.

Earlier study on the leaf extracts of the *Ventilago denticulata* Willd. showed strong antioxidant activity[14]. The phenolic constituents, including antraquinones, ventinone A and B, lupeol, beta-sitosterol and its glucoside. Moreover, the ethanolic extract of plant also show anti-inflamation and anti-microbial activites[11].

The antioxidant activity of an extract, especially when dealing with food and biological sample depends on several parameters such as the matrix effect, the oxidation parameters and the antioxidant reaction conditions. In order to evaluate the antioxidant activity of a natural product, it is crucial to implement more than one antioxidant methods, taking into consideration the various oxidation aspects in the systems under scrutiny. In this study, antioxidant activity of Rhang dang leaves extract have been determined by FRAPs assay. For the measurements of the reducing ability, the result showed high potency of reducing power found in the ethanol extract of Rhang dang leaves. The

results of the current study confirm our previous report of strong antioxidant activity in Rhang dang leaves[14].

Oxidative response regulates many physiological processes in human health. But if not properly regulated, it could also lead to a number of deleterious effects. ROS occur as natural by-products of oxygen metabolism and have intracellular functions. Excessive ROS can disrupt the intracellular homeostasis of redox system and alter cell function via oxidative damage[15]. Direct evolution of ROS yields is a good indicator of oxidative damage to living cell. In this study hydrogen peroxide was utilized to induce oxidative stress in HepG2 cells. It increased the fluorescence intensity in DCFH-DA loaded HepG2 cells, indicating an increase in the generation of intracellular ROS. After pre-treatment with Rhang dang leaves for 24 hr before exposed to an oxidative stress, the result shows that the natural antioxidants of plant extract strongly decrease the steady-state generation of ROS by HepG2 cell, thus preventing or delaying conditions which favor oxidative stress in the cell. These data suggest that increased levels of ROS generated during the oxidative stress period are being more efficiently quenched in cells pretreated with plant extract, resulting in a reduced cell oxidative damage.

Iron is an element essential for all forms of life because of its role in major biological processes such as the tricarboxylic acid cycle, electron transport, nitrogen fixation, DNA synthesis and detoxification reaction[16,17]. However, Iron overload caused by increased iron absorption and multiple transfusions is commonly associated with oxidative stress in thalassemia major patients[18]. Excess free iron as ferrous iron (Fe^{2+}) is toxic and participates in Haber-Weiss and Fenton reactions to catalyze the conversion of superoxide to hydrogen peroxide and the hydrogen peroxide to highly reactive hydroxyl radicals. Under iron overload condition, labile iron pool (LIP) represents the non-ferritin-bound, redox-active iron which is implicates in oxidative stress and cell injury[19]. The intracellular iron pool plays a role in generation of free radicals and is thus the target of chelators used for the treatment of iron overload. The nature of LIP was also revealed by its capacity to promote formation of reactive oxygen species (ROS), whether from endogenous or exogenous redox-active sources. Presumably, LIP and ROS levels follow similar “rise and fall” patterns as a result of changes in iron import versus iron chelation, or ferritin degradation versus ferritin synthesis[20]. Our present results clearly show that both ethanolic and aqueous extracts of Rhang dang leaves interacted with iron ion and reduce LIP in iron-loaded HepG2 cells.

In conclusion, the investigation of Rhang dang leaves extract showed that it possesses both reducing power and iron chelating activity and reduces intracellular ROS. Taken together, the current finding will be of use in elucidating the pharmacology an application of Rhang dang leaves as a potential iron chelating drug in the treatment of iron overload disease.

What is already known on this topic?

The ethanolic extract of Rang dang leaves exhibited strong antioxidant activity and prevented hemolysis, but the iron chelating activity remains unknown.

What this study adds?

The present study was performed to assess reducing power and iron chelating activity of Rhang dang leaves extract. Rhang dang leaves extract showed that possesses both reducing power and iron chelating activity in both of in vitro and iron loaded hepatocytes.

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Potential conflicts of interest

None.

References

1. Bancirova M, Lasovsky J. The photodynamic effect: the comparison of chemiexcitation by luminol and phthalhydrazide. Luminescence 2011; 26: 410-5.
2. Riegel RE, Valvassori SS, Elias G, Reus GZ, Steckert AV, de Souza B, et al. Animal model of mania induced by ouabain: Evidence of oxidative stress in submitochondrial particles of the rat brain. Neurochem Int 2009; 55: 491-5.
3. Prakash D, Suri S, Upadhyay G, Singh BN. Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. Int J Food Sci Nutr 2007; 58: 18-28.
4. Gohil K, Moy RK, Farzin S, Maguire JJ, Packer L. mRNA expression profile of a human cancer cell line in response to Ginkgo biloba extract: induction of antioxidant response and the Golgi system. Free

Radic Res 2000; 33: 831-49.
5. Morel I, Lescoat G, Cogrel P, Sergent O, Pasdeloup N, Brissot P, et al. Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. Biochem Pharmacol 1993; 45: 13-9.
6. Verga Falzacappa MV, Muckenthaler MU. Hepcidin: iron-hormone and anti-microbial peptide. Gene 2005; 364: 37-44.
7. Kakhlon O, Gruenbaum Y, Cabantchik ZI. Repression of ferritin expression increases the labile iron pool, oxidative stress, and short-term growth of human erythroleukemia cells. Blood 2001; 97: 2863-71.
8. Kakhlon O, Gruenbaum Y, Cabantchik ZI. Ferritin expression modulates cell cycle dynamics and cell responsiveness to H-ras-induced growth via expansion of the labile iron pool. Biochem J 2002; 363: 431-6.
9. Sudan R, Bhagat M, Gupta S, Singh J, Koul A. Iron (FeII) chelation, ferric reducing antioxidant power, and immune modulating potential of Arisaema jacquemontii (Himalayan Cobra Lily). Biomed Res Int 2014; 2014: 179865.
10. Sumalnop U. The chemical constituents of the leaves of "rhamnaceae ventilago calyculata ful" [Thesis]. Bangkok: University of Medical Sciences; 1968.
11. Sornprasert R, Norseangsri P, Hambananda A. Effect of crude extracts from Rang Daeng(Ventilago denticulata willd) leaves on growth of Colletotrichum gloeosporioides and Fusarium oxysporum. Rajabhat J Sci Humanit Soc Sci 2013; 13: 42-7.
12. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239: 70-6.
13. Moneim AE. Oxidant/Antioxidant imbalance and the risk of Alzheimer's disease. Curr Alzheimer Res 2015; 12: 335-49.
14. Pongjanta A, Pangjit K, Srichairatanakool S. Antioxidant Activity and Cytotoxic Effect of Ventilago denticulata Willd Leaves Extracts. J Med Assoc Thai 2016; 99 (Suppl 1): S51-7.
15. Aruoma OI, Kaur H, Halliwell B. Oxygen free radicals and human diseases. J R Soc Health 1991; 111: 172-7.
16. Deng J, Cheng J, Liao X, Zhang T, Leng X, Zhao G. Comparative study on iron release from soybean (Glycine max) seed ferritin induced by anthocyanins and ascorbate. J Agric Food Chem 2010; 58: 635-41.
17. Zhang Y, Mikhael M, Xu D, Li Y, Soe-Lin S, Ning B, et al. Lysosomal proteolysis is the primary degradation pathway for cytosolic ferritin and cytosolic ferritin degradation is necessary for iron exit. Antioxid Redox Signal 2010; 13: 999-1009.
18. Laksmitawati DR, Handayani S, Udyaningsih-Freisleben SK, Kurniati V, Adhiyanto C, Hidayat J, et al. Iron status and oxidative stress in beta-thalassemia patients in Jakarta. Biofactors 2003; 19: 53-62.
19. Konijn AM, Glickstein H, Vaisman B, Meyron-Holtz EG, Slotki IN, Cabantchik ZI. The cellular labile iron pool and intracellular ferritin in K562 cells. Blood 1999; 94: 2128-34.
20. Kruszewski M. Labile iron pool: the main determinant of cellular response to oxidative stress. Mutat Res 2003; 531: 81-92.

คุณสมบัติในการรีดิวซ์ การจับธาตุเหล็กและการขจัดอนุมูลอิสระของสารสกัดจากใบรางแดงในเซลล์ตับเพาะเลี้ยงที่มีภาวะเหล็กเกิน

อนุสรา พงค์จันตา, กาญจนา แปงจิตต์, สมเดช ศรีชัยรัตนกูล

ภูมิหลัง: *ภาวะเหล็กเกินพบในผู้ป่วยธาลัสซีเมียชนิดบีตาที่ได้รับการเติมเลือดเป็นประจำ ปริมาณธาตุเหล็กมากเกินสามารถเร่งการสร้างอนุมูลอิสระ ซึ่งนำไปสู่การทำลายสารชีวโมเลกุลและการทำหน้าที่ผิดปกติของอวัยวะต่าง ๆ ดังนั้นการลดภาวะเหล็กเกินจึงเป็นแนวทางหนึ่งในการรักษาโรคที่เกี่ยวข้องกับสภาวะเครียดที่เกิดขึ้นในร่างกาย*

วัตถุประสงค์: *ศึกษาคุณสมบัติของสารสกัดรางแดงในการรีดิวซ์และการจับธาตุเหล็กในหลอดทดลองและฤทธิ์ในการลดอนุมูลอิสระ ในเซลล์ตับเพาะเลี้ยงที่มีภาวะเหล็กเกิน*

วัสดุและวิธีการ: *นำสารสกัดหยาบใบรางแดงด้วยน้ำและเอธานอลมาทำการวิเคราะห์การรีดิวซ์ การกำจัดอนุมูลอิสระ การจับธาตุเหล็กและการลดปริมาณธาตุเหล็กในเซลล์ตับ*

ผลการศึกษา: *สารสกัดจากรางแดงด้วยเอธานอลมีคุณสมบัติในการรีดิวซ์ที่สูง สามารถจับธาตุเหล็กและยับยั้งการเกิดอนุมูลอิสระได้ โดยสามารถป้องกันการเหนี่ยวนำให้เกิดอนุมูลอิสระจากไฮโดรเจนเปอร์ออกไซด์ในลักษณะสัมพันธ์กับความเข้มข้นที่เพิ่มขึ้น อีกทั้งสารสกัดจากรางแดงยังสามารถลดปริมาณธาตุเหล็กในเซลล์ตับเพาะเลี้ยงที่มีภาวะเหล็กเกินได้ดี*

สรุป: *สารสกัดรางแดงมีคุณสมบัติในการรีดิวซ์อนุมูลอิสระการจับธาตุเหล็ก และการป้องกันเซลล์จากภาวะเครียด ซึ่งคุณสมบัติเหล่านี้อาจเกิดจากสารสำคัญในรางแดงที่มีความสามารถในการลดภาวะเหล็กเกินที่จะเหนี่ยวนำให้เกิดความเป็นพิษด้วยเหตุนี้ อาจจะมีประโยชน์ในการพัฒนาเป็นยาจับธาตุเหล็กสำหรับผู้ป่วยที่มีภาวะเหล็กเกินต่อไป*