The Comparative Study of the Protective Effect on Human Oxidative Hemolysis of Polyphenol Extracts from Tea Seed Oil and Olive Oil

Nuchanart S [a*], Panadda R[a], Rattana T[a], Jirada S[b] and Intira T[c]

[a] *Faculty of Medicine, Thammasat University, Pathumthani, 12120, Thailand*
[b] *Faculty of Science and Technology, Thammasat University, Pathumthani, 12120, Thailand*
[c] *Faculty of Science, Rangsit University, Pathumthani, 12000, Thailand*

*Corresponding author email: sunuchan@tu.ac.th
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ABSTRACT: Antioxidants are known to have the capacity to eradicate free radicals believed to be the products of human oxidative stress associated with human diseases. Olive oil is a one type of antioxidant particularly its polyphenols that shows antioxidant activity, unfortunately, its consumption is only affordable to some people. Tea seed oil was observed to have a composition that had the properties similar to olive oil but no scientific work comparing any antioxidative property between these two cooking oils were reported. Thus, this study aims to investigate the protective effect of polyphenols contained in tea seed oil and compare them with olive oil. The polyphenols were extracted from oils by sequential solvent extraction (methanol, petroleum ether and ethyl acetate) for the study its protective effect on human oxidative hemolysis induced by 1.3 M hydrogen peroxide using a 3-groups design (control, hydrogen peroxide and test compounds). A comparative study of each group was recorded, as absorbance of supernatant hemolysis fraction, before and after hydrogen peroxide induction. The results showed that IC_{50} of polyphenol extract (PE) from tea seed oil was less than the PE from olive oil indicating that PE from tea seed oil has more antioxidative potency than olive oil in the oxidative hemolysis model and this may be useful to prevent diseases associated with oxidative stress.

Keywords: Antioxidant, hemolysis, olive oil, polyphenols, tea seed oil

Introduction

Free radicals produced by oxidative stress are known to be associated with human diseases such as cardiovascular diseases (Aviram, 2000; Griendling and Fitz Gerald, 2003), diabetes mellitus (Griendling and Fitz Gerald, 2003; Wolff, 1993) and cancer (Trueba *et al.*, 2004). To reduce the risk of these diseases, the consumption of the natural compounds, acting as antioxidant agents from various sources such as fruits and vegetables are well known nowadays as a very essential supplement to a diet. Moreover, using antioxidative cooking oil is another potential way to prevent risk. One of the cooking oils is olive oil.

Olive oil from the olive tree (*Olea europaea*) is preferred as a valuable cooking oil in households around the world. It has been reported that the polyphenol compounds in olive oil (such as hydroxytyrosol and oleuropein) have antioxidative activities (D'Angelo *et al.*, 2005; Edgecombe *et al.*, 2000; Romero *et al.*, 2007) to inhibit low-density lipoprotein (LDL) oxidation (Visioli *et al.*, 1995), reduce platelet aggregation (Petroni *et al.*, 1995) as well as to reduce the incidence of atherosclerosis. However, the consumption of olive oil is limited only to the high-end consumer because of its relatively high price. Comparatively, tea seed oil (*Camellia oleifera*) is recently attracting attention due to its polyphenol composition believed to have antioxidative effects similar to olive oil. However, there was no scientific report comparing the antioxidative properties between these two cooking oils. The aim of this study was therefore to determine both, the amount of polyphenols, and the protective effect on human oxidative hemolysis induced by hydrogen peroxide contained in tea seed oil when compared with olive oil.

Materials and Methods

Polyphenol extraction from tea seed oil and olive oil

Tea seed oil (Naturel, Thailand) and extra virgin olive oil (Bertolli, Italy) purchased from a department store were used as the samples in this study for polyphenol extraction (liquid-liquid extraction) using methanol, petroleum ether and ethyl acetate, respectively (Cui *et al.*, 2006). In brief, after mixing the samples with the requisite solvents described above (1:1 ratio), the following steps i.e. stirring, centrifugation and evaporation

were repeated for each extraction. Polyphenol extract (PE) from the final ethyl acetate fraction was finally kept at -20°C during the experiments.

Protective effects of the PE against oxidative hemolysis of human erythrocytes

i) Erythrocyte extraction

Erythrocytes (RBCs) from healthy volunteers (n=35) were extracted by the modified method as described by Ko *et al.* (Ko *et al.*, 1997). After precipitating and washing with phosphate-buffered saline glucose (PBSG), 10% cell suspension in PBSG was prepared for oxidative stress induction.

ii) Oxidative stress induction

PE from ethyl acetate fraction was further investigated for its protective effects on the human oxidative hemolysis, induced by H_2O_2. The study was designed by dividing the experiments into three groups; a negative control (RBCs in PBSG), a positive control (1.3 M H_2O_2-treated RBCs in PBSG) and test groups (1.3 M H_2O_2-treated RBCs in PBSG in the presence of the PE from tea seed oil or olive oil). All the groups of experiments were conducted at 37°C for 3 h. The extent of hemolysis was determined by measuring the absorbance at 540 nm. All experiments were duplicated.

The percent inhibition of hemolysis was calculated according to the following equation:

$$\%\,\text{Inhibition} = \frac{[(B-A)-(C-A)]}{(B-A)} \times 100$$

where A, B and C are the absorbance values of a negative control, a positive control and test compound at 540 nm.

Statistical analysis

The data was reported by mean ± standard deviation (SD). The comparative data was analyzed using one-way ANOVA and $P < 0.05$ was considered significant.

Results

Polyphenol extraction from tea seed oil and olive oil

After subsequent liquid-liquid extraction, the polyphenol extraction yields from tea seed oil and olive oil were 50.00 ± 9.52 and 485.00 ± 79.70 mg per liter of oil, respectively.

Protective effects of the PE against oxidative hemolysis of human erythrocytes

1.3 M H_2O_2-induced oxidative stress resulted in hemolysis of erythrocytes. The maximal hemolysis effect was observed at 15 min after oxidative induction. Moreover, no significant difference ($P > 0.05$) was found when prolonged treatment was induced (15-180 min). Interestingly, H_2O_2 treated RBCs group in the presence of the PE obtained from both tea seed oil and olive oil (0.001-10 µg/ml) showed a significantly lower absorbance values ($P < 0.05$) than that of H_2O_2 treated RBCs group (**Figure 1**).

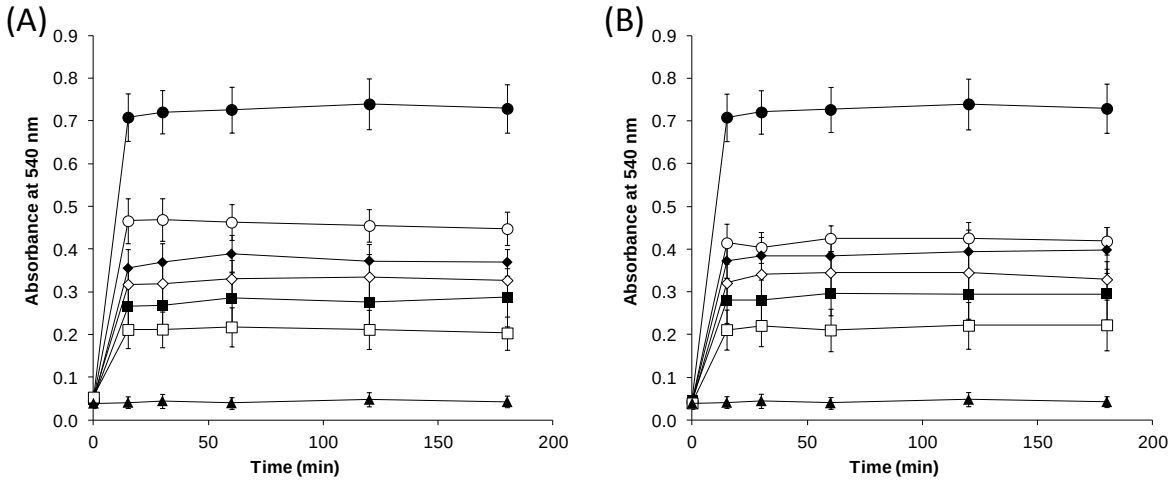

Figure 1: Change in absorbance values in the presence of the PE from different oils: (A) tea seed oil; (B) olive oil. ●:1.3 M H_2O_2-treated RBCs in PBSG; ▲: RBCs in PBSG; ○, ♦, ◊, ■ and □: 1.3 M H_2O_2-treated RBCs in PBSG in the presence of 0.001, 0.01, 0.1, 1 and 10 µg/ml PE from oil, respectively. Values are the mean ± SD (n=35).

At the end of the incubation period (3 h), the percentage of hemolysis inhibition of the PE was calculated. The results showed the percentage of inhibition increased in a concentration-dependent manner in the presence of the PE. The protective potencies of the PE from tea seed oil and olive oil were compared using a concentration that produced 50% hemolysis inhibition (IC_{50}). It was found that an IC_{50} of PE from tea seed oil was less than the PE from olive oil (**Table 1**).

Table 1: The percentage of hemolysis inhibition of the PE from tea seed oil and olive oil at 3 hr after oxidative induction.

PE	Concentration (µg/ml)					IC_{50} (ng/ml)
	0.001	0.01	0.1	1	10	
Tea seed oil	40.05 ± 12.96^{a}	$51.04\pm12.47^{b,c,d}$	$58.21\pm12.26^{d,e}$	$64.14\pm16.38^{e,f}$	76.34 ± 9.19^{g}	9.16
Olive oil	$43.95\pm11.96^{a,b}$	$47.41\pm14.34^{a,b,c}$	$57.16\pm13.47^{c,d,e}$	62.55 ± 15.94^{e}	$73.47\pm16.77^{f,g}$	18.00

Values are the mean ± SD (n=35).
Different superscript letters are significantly different ($P<0.05$).

Discussion

The polyphenols that were extracted from tea seed oil were those reported to have important composition in tea seed oil (He *et al.*, 2011). Note that various polyphenols in olive oil were also reported (D'Angelo *et al.*, 2005; Edgecombe *et al.*, 2000; Manna *et al.*, 1997; Manna *et al.*, 1999; O'Dowd *et al.*, 2004; Romero *et al.*, 2007). Our results showed that the amount of polyphenol extraction yields from tea seed oil was approximately 10 times lower than that from olive oil (note: PE from tea seed oil = 50.00 ± 9.52 mg per liter of oil; PE from olive oil = 485.00 ± 79.70 mg per liter of oil). The difference in polyphenol amounts in the oil products could be due to different oil production processes.

The protective effects of the PE on oxidative hemolysis were investigated and the results showed the PE obtained from both tea seed oil and olive oil displayed antioxidant activity by hemolysis prevention. All concentrations of PE used in these experiments (0.001-10 µg/ml) showed the abilities against oxidative hemolysis of human erythrocytes consistent with previous studies (Anesini *et al.*, 2008; Fazel *et al.*, 2009; Youdim *et al.*, 2000). The results of antioxidative effect of the PE from tea seed oil support the findings that the methanolic extract from tea seed oil had an antioxidative effect by determination of diphenylpicrylhydrazyl (DPPH) scavenging activity and Trolox equivalent antioxidant capacity (TEAC) (Lee and Yen, 2006).

When the efficacy comparison of hemolysis prevention between the PE from tea seed oil and olive oil was considered, our results show that there was no significant difference in the percentage of hemolysis inhibition between the two oils at any given time ($P > 0.05$). Nonetheless, at the end of the incubation period, it was found that an IC_{50} of PE from tea seed oil (IC_{50} = 9.16 ng/ml) was less than that of PE from olive oil (IC_{50} = 18.00 ng/ml), suggesting that the PE from tea seed oil had a greater protective effect against hemolysis of erythrocytes.

The erythrocytes in this study were induced by H_2O_2 for hydroxyl radical production. This radical affected hemoglobin in erythrocytes and caused the degradation to form Heinz's bodies, which destroyed the membrane of erythrocytes, causing hemolysis (Beutler, 2006; Bunn and Rosse, 2005; van den Berg *et al.*, 1992). It was found that PE obtained from both tea seed oil and olive oil showed an antioxidative effect and possesses hemolysis preventative ability. The antioxidative mechanism of PE from tea seed oil may be divided into two possible mechanisms; i.e the decrease of H_2O_2; and the decrease of the hydroxyl radical, which should be investigated in the future. However, this study demonstrated the antioxidative effects of PE from tea seed oil and olive oil were likely to have the properties to prevent or reduce the free radicals that cause tissue damage and may reduce the incidence of diseases associated with free radicals (**Figure 2**).

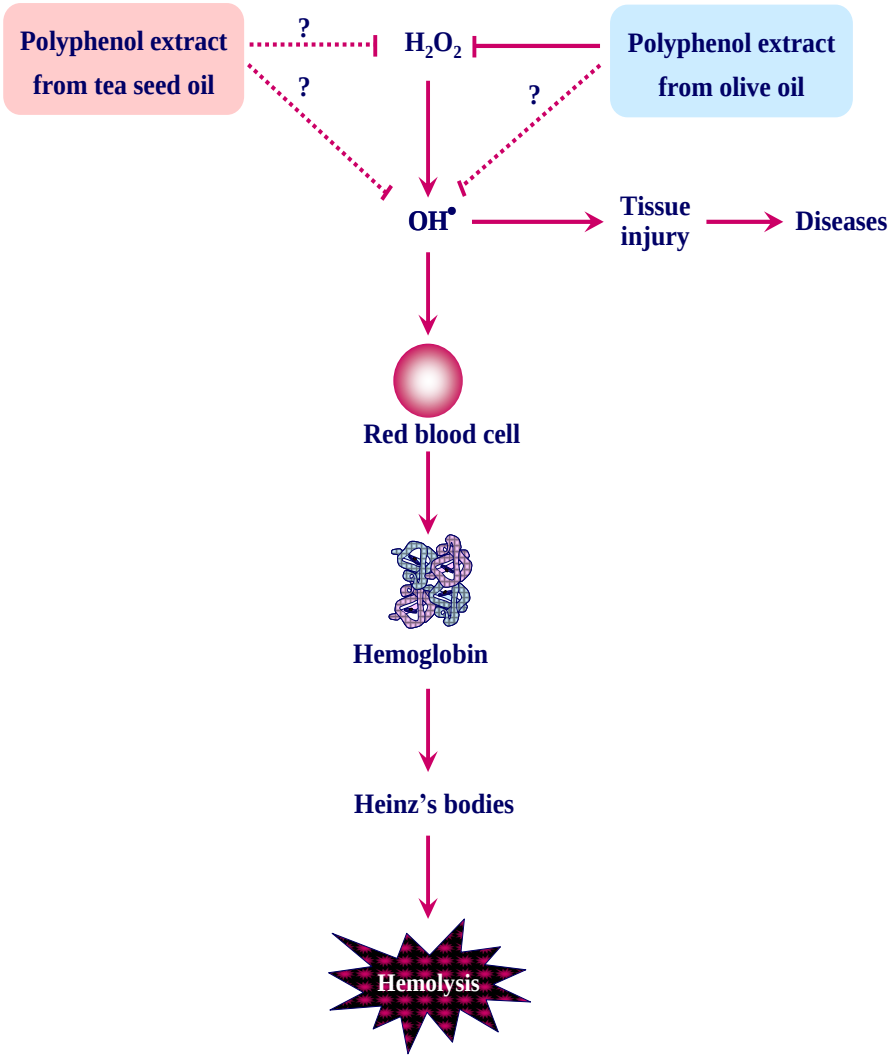

Figure 2: The possible antioxidative mechanisms of the PE from tea seed oil and olive oil in oxidative hemolysis model using H_2O_2 induction.

The possibility of using alternative oil other than olive oil as a common cooking oil to prevent diseases associated with oxidative stress, such as cardiovascular disease, diabetes mellitus and cancer may be suggested. Tea seed oil is a good option in addition to its a higher smoking point (252°C) compared to olive oil (161°C) (Emerald Harvest Inc., 2007).

The polyphenol compounds vary. Polyphenols in olive oil which have antioxidative activity include hydroxytyrosol and oleuropein (D'Angelo *et al.*, 2005; Edgecombe *et al.*, 2000; Romero *et al.*, 2007). Hydroxytyrosol had an antioxidative capacity by scavenging hydrogen peroxide but not superoxide anion in human neutrophils (O'Dowd *et al.*, 2004). Polyphenol compounds found in tea tree oil such as catechin, flavonol, phenolic acid and depside were reported to have antioxidative activities (Andlauer *et al.*, 1999; He *et al.*, 2011). Catechin content in a polyphenol extract from tea seed oil was recently quantified to be 85.51µg/mg by using HPLC method (Suealek *et al.*, 2012). Therefore, further characterization of polyphenols in tea seed oil and their antioxidative activities is warranted.

Conclusion

It was concluded that the PE obtained from both tea seed oil and olive oil have protective effects on human oxidative hemolysis. Although the polyphenol extraction yields from tea seed oil was less than olive oil, the polyphenols from tea seed oil has more antioxidative potency than olive oil in the oxidative hemolysis model. In the future, the comparison of clinical effects between tea seed oil

and olive oil should be further investigated in order to learn more about their benefits for human health.

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