Antioxidant and anti-inflammatory activities of durian and rambutan pulp extract

Chingsuwanrote, P., Muangnoi, C., Parengam, K. and Tuntipopipat, S.

Institute of Nutrition, Mahidol University, Salaya, Putthamonthon, Nakhonpathom, Thailand

Abstract

Durian (Durio zibethinus murr.) and rambutan (Nephelium lappaceum) are popular exotic fruits grown and consumed in Southeast Asia. Two economically important cultivars of durian and rambutan were studied to assess their antioxidant and anti-inflammatory activities. Non-differentiated U937 monocyte-like cells were pre-treated with non-toxic doses of ethanol extract from fruit pulp prior to inducing oxidative stress with \( \text{H}_2\text{O}_2 \). Antioxidant capacity of fruit extracts was measured from the suppressive effect on reactive oxygen species (ROS) formation. Anti-inflammatory activity of non-toxic extracts doses also was assessed by measuring secretion of cyto/chemokines into medium of lipopolysaccharide-induced differentiated U937 cells treated with fruit extracts. Durian extracts were more potent at suppressing ROS formation and decreasing secretion of tumor necrosis factor alpha (TNF-\( \alpha \)) and interleukin-8 (IL-8) than rambutan extracts. The extract from the Monthong cultivar of durian possessed greater antioxidant and anti-inflammatory activities than that prepared from the Chanee cultivar. Extract from the cultivar Sichompu of rambutan inhibited ROS formation but the extract from Rongrien had no significant activity. Both rambutan cultivars inhibited secretion of TNF-\( \alpha \), but not IL-8 secretion. The results indicate that durian pulp has greater potential for the development of functional foods than rambutan. An in vivo study is now needed to confirm these biological activities.

Introduction

Increased consumption of fruit and vegetables is associated with a lower risk of all causes of mortality, and particularly cardiovascular disease (Wang et al., 2014). Inflammation plays a key role in the initiation and progression of cardiovascular disease (Libby et al., 2002). Atherosclerosis is a disease in which plaque builds up inside arteries. Plaque is formed by cholesterol, fatty substances, cellular waste products, calcium and fibrin. Plaque may block the blood’s flow through an artery in various vital organs including the heart. Some diseases may develop from atherosclerosis such as coronary heart disease, angina, carotid artery disease, peripheral artery disease and chronic kidney disease (Golia et al., 2014). Atherosclerotic plaque is enriched with leukocytes, and macrophages are the major immune cells involving in the development of atherosclerosis. Many macrophage-derived pro-inflammatory mechanisms associated with atherogenesis have been characterized, such as cell adhesion, cytokines/chemokine production and protease secretion which are specifically targets for treating patients with atherosclerosis (Martynowicz et al., 2014; Mendel et al., 2015). In addition, during chronic inflammation, endogenous antioxidant capacities are exceeded due to continuous production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS/RNS molecules are highly reactive and can interact with lipids, proteins, nucleic acids, carbohydrates, and small molecule metabolites (Miki and Funato, 2012). These biomolecules promote oxidation, nitrosation, and nitration which in turn provoke inflammatory responses (Churchii and Maccarrone, 2011). Lower concentrations of antioxidants are present in patients with cardiovascular disease (Murr et al., 2009), whereas markers of oxidative stress in serum are elevated (Fuchs et al., 2009) due to increased demand from excessive generation of ROS by activated macrophages. Low intake of antioxidant-rich diets may further contribute to the risk and extent of the pathology. Thus, increased intake of dietary antioxidants represents one strategy to decrease disease-related oxidative stress.

Fruit and vegetables are well-known excellent sources of polyphenolic compounds and particularly flavonoids. Fruit polyphenols have a wide range of health promoting activities including antioxidant (Xia et al., 2010), anti-inflammatory (Joseph et
al., 2015), anti-diabetic (Babu et al., 2013), anti-obesity (Wang et al., 2014), anti-tumor (Jurikova et al., 2012) and anti-Alzheimer’s disease (Malar and Devi, 2014). Several exotic fruits including durian, mangosteen and rambutan are rich not only nutrients but also bioactive compounds (Gorinstein et al., 1999; Haruenkit et al., 2007; Fila et al., 2012; Johnson et al., 2013; Charoenkiatkul et al., 2015; Ho and Bhat, 2015). Durian (Durio zibethinus murr.) is known as a king of fruits in Southeast Asia. Bioactive compounds such as anthocyanins, flavonoids, carotenoids, and flavanols have been reported to be present in durian pulp (Haruenkit et al., 2010; Gorinstein et al., 2011). Several cultivars of durian are grown in Thailand (http://durianinfo.blogspot.com/p/blog-page_18.html) and different cultivars at the same maturity and ripening have varied concentrations of bioactive compounds and antioxidant capacity (Toledo et al., 2008). The fruit, hull, leaves and roots of durian have been traditionally used to treat many diseases (Ho and Bhat, 2015). Rambutan (Nephelium lappaceum) is another exotic fruit in Southeast Asia that is consumed fresh, canned or processed. Rambutan pulp has been reported to possess high abundant of carbohydrates, fats, proteins, phosphorus, iron, vitamin C, calcium and flavonoids (Fila et al., 2012; Johnson et al., 2013). Due to their high abundance of nutrients and active compounds, the anti-inflammatory and cellular antioxidant activity of durian and rambutan pulp warrants investigation.

The purpose of the present study was to assess antioxidant and anti-inflammatory activities of extracts prepared from two popular cultivars of durian, (Monthong and Chanee) and two popular cultivars of rambutan, (Sichompu and Rongrien). Antioxidant capacity of the extract was assessed using promonocytic non-differentiated U937 cells exposed to hydrogen peroxide and anti-inflammatory activity was determined in cultures of differentiated U937 cells activated with lipopolysaccharide (LPS).

Materials and Methods

Chemicals

Roswell Park Memorial Institute 1640 medium (RPMI-1640), LPS (Escherichia coli O11:B4), Phorbol 12-Myristate 13-Acetate (PMA) and N-acetyl-L-cysteine (NAC) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Penicillin and streptomycin were obtained from Invitrogen (Grand Island, NY, USA). Fetal bovine serum (FBS) was obtained from PAA Laboratories (Haidmannweg, Austria). Human TNF-α, IL-6, IL-8 and MCP-1 capture and biotin-labeled detection antibodies were purchased from Peprotech Inc. (Rocky Hill, NJ, USA). Other chemicals were analytical grade.

Preparation of extract

Figure 1 showed the ripened Chanee and Monthong cultivars of durian (Durio zibethinus murr.) and Sichompu and Rongrien cultivars of rambutan (Nephelium lappaceum Linn) which were purchased from three local markets in Nakhornpathom province (central region of Thailand). The pulp of each fruit was collected from each market and combined to obtain a composite sample. Pulp was homogenized with a commercial blender (Moulinex, Thailand) and lyophilized. Dry pulp was packed in a vacuo-aluminum foil and stored at -20°C until use. The extraction protocol was modified from that of Jayakumar and Kanthimathi (2011). The dry pulp (1.0 g) was extracted with 30 mL of 90% ethanol by vigorously mixing for 2 min and sonicated in an ultrasonic bath for 10 min at room temperature. The extraction procedure was repeated. Homogenized samples were centrifuged (7500 g, 10 min, room temperature), supernatants were combined and solvent was evaporated under vacuum at 42°C until dry. The dry film was solubilized in phenol red-free, serum-free RPMI medium. The final concentration was diluted to designated concentrations with phenol-red free, serum free RPMI medium and membrane filtered with 0.2 µm pores prior to addition to cell cultures.
Cell culture

The human promonocytic U937 cells were obtained from ATCC (Bethesda, MD, USA). Cells were grown in complete medium containing RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 mg/mL of streptomycin at 37°C in humidified atmosphere of 5% CO2/95% air. Spent medium was replaced with fresh medium every other day.

Intracellular reactive oxygen species (ROS)

Promonocytic non-differentiated U937 cells at 2 × 10⁶ cells in 1 mL complete phenol red free medium were incubated with an equal volume of medium containing either 10-40 mg/mL of fruit extract or 10 mM NAC (positive control) in 6 well dishes. After 2 h, dishes were centrifuged (1,000 g for 10 min time at 25°C) and the cell pellet was suspended in basal RPMI-1640 phenol red free medium containing 20 µM 2, 7-dichlorofluorescein diacetate (DCFH-DA) and incubated at 37°C for 30 min. Cell pellet after centrifugation (1,000 g for 10 min) was suspended and adjusted to contain 2 × 10⁶ cells/mL in complete phenol red-free medium. An aliquot (50 µL) was incubated with an equal volume of basal medium or basal medium containing 400 µM H2O2 in blackened 96 well plate for 20 min. Fluorescence at an excitation wavelength of 485 nm and emission wavelength at 530 nm was monitored.

Cytotoxicity of fruit extract on non-differentiated U937 cells

Cytotoxicity of fruit extract was assessed by sulforhodamine B (SRB) assay (Vichai and Kirtikara, 2006). After measuring the fluorescent signal in the previous section, the suspensions were centrifuged (1,000 g for 10 min) and collected cells were suspended in 100 µL/well of cold PBS. Cellular protein was precipitated by adding of 20 µL/well of 50% trichloroacetic acid and incubated at 4°C at for 2 h. The fixed cells were extensively washed with distilled water and stained with 0.1% SRB for 20 min before solubilizing with 10 mM Tris-hydro-methyl-aminomethane at pH 10. Absorbance at 500 nm was measured. The absorbance of control cells not exposed to the extract was defined as 100% viable.

Inflammatory cyto/chemokines

Promonocyte U937 cells were induced to differentiate to macrophages (D-U937) by incubating 1 × 10⁶ cells/mL in complete medium containing 20 ng/mL of PMA for 48 h (Okoko and Oruambo 2009). D-U937 were suspended and reseeded at 1 × 10⁶ cells/mL in 24-well plates in serum- and phenol red-free RPMI 1640. The cells were treated with 0.05-0.2 mg/mL of fruit extract or 10 mM NAC in serum-and phenol red free RPMI-1640 for 2 h prior to activation with 10 ng/mL LPS for 18 h. Spent medium was collected to quantify TNF-α, IL-6, IL-8 and MCP-1 by ELISA as described previously (Tuntipopipat et al., 2011). The cytotoxicity of fruit extracts on D-U937 was assessed according to the protocol described in the previous section.

Statistical analysis

SPSS version 16 was used for statistical analyses. All parameters were conducted in triplicate and each experiment was performed at least 2 times. The descriptive statistics including mean and SE were calculated for ROS, inflammatory cyto/chemokines and percent inhibition. Means were analyzed by one-way ANOVA when appropriate following with Tukey’s multiple comparisons. Differences were considered significant at p<0.05 were.

Results and Discussion

Effect of pulp extract from durian and rambutan on ROS formation

Viability of promonocytic non-differentiated U937 cells was not affected during incubation in medium containing 10-40 mg/mL of ethanolic extract prepared from the pulp of ripe durian and rambutan (Figure 2A and 3A). Exposure of the non-differentiated U937 cells to 400 µM of H2O2 for 20 min increased production of ROS 5-fold over the basal level (Figure 2B and 3B). N-acetyl cysteine (NAC), a thiol compound, with well documented antioxidant activities in vivo and in vitro studies (Sadowska et al., 2007) was used as a positive control to determine the antioxidant activities of the fruit extracts. Pre-treatment of non-differentiated U937 cells with NAC reduced H2O2-induced ROS production by 34% - 41% (Figure 2B and 3B). Pre-treatment of non-differentiated U937 cells with 40 mg/mL and 20 mg/mL extract from the pulp of the Monthong cultivar of durian reduced H2O2-induced ROS formation by 30% and 18%, respectively (p<0.05) (Figure 2B). Pre-treatment of the non-differentiated U937 cells with 40 mg/mL of extract of the pulp from Chanee decreased H2O2-induced production of ROS by 21% (p<0.05) (Figure 2B) while the lower concentrations of this extract failed to significantly alter generation of ROS. These results suggested that pulp from the Monthong cultivar of durian contained a greater concentration of antioxidant compounds than that of the Chanee cultivar. Antioxidant capacity of durian
is dependent on the ripening stage. Ripe durian was reported to contain relatively high concentrations of polyphenols and flavonoids compared to mature and overripe durians (Leontowicz et al., 2007). Pulp of the Monthong cultivar contained greater amounts of total polyphenol and flavonoids than the Chanee cultivar at the same stage of ripening (Leontowicz et al., 2008).

Total polyphenol and flavonoid content of ethanol extract from durian pulp was well correlated with antioxidant capacity as determined by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay (Leontowicz et al., 2008). The antioxidant and anti-inflammatory activities of dietary polyphenols and flavonoids has been extensively documented (Marzocchella et al., 2011; Agati et al., 2012; Harasym and Oledzki, 2014; Joseph et al., 2015). The observed antioxidant activity of ethanolic extracts of ripe pulp from the Monthong and Chanee cultivars of durian in our study are well aligned with those of Leontowicz et al. (2008).

Pre-treatment of non-differentiated U937 cells with 40 mg/mL of extract from rambutan cultivar the Sichompu inhibited ROS formation by 25% (p<0.05) (Figure 3B), whereas the extract from cultivar the Rongrien lacked significant activity. The antioxidant activity of rambutan was previously studied for extracts prepared from peel and leaf (Palanisamy et al., 2008; Thitilertdecha et al., 2008; Khonkarn et al., 2010; Sun et al., 2012). DPPH-scavenging activity of extract from rambutan fruit pulp (Palanisamy et al., 2008) which was relatively low compared with those obtained from its peel and leaf. Ascorbic acid, a well-known antioxidant, was reported in fresh and dry rambutan pulp (Johnson et al., 2013). The rambutan pulp also contained some flavonoids (Fila et al., 2012). Both ascorbic acid and flavonoids in rambutan pulp may have contributed to suppressed ROS formation in the present study.

**Effect of pulp extract from durian and rambutan on secretion of cyto-chemo-kines by macrophage-like U937 cells**

To investigate the anti-inflammatory effects of durian and rambutan extracts on PMA-differentiated
U937 cells with macrophage-like phenotype (D-U937 cells), these cells were treated with 0.05-0.2 mg/mL of durian or rambutan extract or 10 mM NAC for 2 h prior to further incubation without or with LPS for another 18 h. Viability of LPS-induced D-U937 cells was not significantly affected by co-incubation with either of the extracts (data not shown). Secretion of TNF-α, IL-8, IL-6 and MCP-1 were significantly increased in D-U937 cells exposed to LPS. Pre-treatment of D-U937 cells with NAC significantly inhibited LPS-induced secretion of TNF-α, IL-8, IL-6 and MCP-1 production (Figure 4 and 5). Tested doses of durian and rambutan pulp extract did not significantly affect LPS-induced IL-6 and MCP-1 secretion by D-U937 cells (Figure 4C-5C and 4D-5D, respectively). Pre-treatment of D-U937 cells with 0.20 mg from Monthong pulp/mL reduced LPS-induced secretion of TNF-α by 21% (Figure 4A), whereas 0.05-0.2 mg extract from Monthong pulp/mL significantly suppressed LPS-induced IL-8 production by 18%-33% (Figure 4B). Pre-treatment of D-U937 cells with 0.2 mg extract from Chanee pulp/mL also significantly decreased secretion of TNF-α by 22% (Figure 4A), while doses of 0.1 and 0.2 mg extract/mL suppressed IL-8 production by 23% and 36%, respectively (Figure 4B). This result indicates that the anti-inflammatory activity of the extract from Monthong pulp was greater than that from the Chanee cultivar of durian. Leontowicz et al. (2008) reported that the pulp of the Monthong cultivar contained higher total polyphenol and flavonoids content than that of Chanee cultivar.
and ripe durian contained a significantly higher total polyphenols, flavonoids, anthocyanins and flavanols than mature and overripe fruits (Arancibia-Avila et al., 2008). Caffeic acid and quercetin were the predominant bioactive compounds identified in pulp from durian (Arancibia-Avila et al., 2008). The anti-inflammatory activity of caffeic acid has been demonstrated in vitro and in vivo. Liu et al. (2008) reported that caffeic acid decreased secretion of IL-8, IL-1β, IL-6, and TNF-α by LPS-activated bovine mammary epithelial cells. Topical treatment with caffeic acid suppressed 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced skin edema by substantially reducing skin thickness myeloperoxidase activity, and several other indicators of histopathology (Zhang et al., 2014). Moreover, topical application of caffeic acid decreased expression of TNF-α, IL-6 and IL-1β.

The antioxidant and anti-inflammatory activities of quercetin also have been reported. Feeding mice diet with 60 mg/kg body weight quercetin for 7 consecutive days ameliorated triglyceride-induced acute liver injury by reducing oxidative stress and inflammatory response (Wang et al., 2015). Quercetin 50 µM also decreased expression of pro-inflammatory mediators IL-6, IL-8, and MCP-1 induced by 4-hydroxynonenal in human retinal pigment epithelium (ARPE-19) cells (Hytti et al., 2015). Pre-treatment peripheral blood mononuclear cells with 10 µM quercetin significantly reduced ochratoxin A-induced generation of nitric oxide (NO), TNF-α, IL-6, and IL-8 (Periasamy et al., 2014). Quercetin fed mice at 60 mg/kg body weight improved survival rate and decreased TNF-α, IL-1β, IL-6, NO, and increased anti-inflammatory cytokine, IL-10 secretion in experimental septic acute lung injury induced by LPS (Wang et al., 2014). These results suggest that both caffeic acid and quercetin in ripe durian pulp likely contributed to the suppression of TNF-α and IL-8 synthesis in the present study. The potential participation of other compounds in the extract from durian pulp merits consideration.

Pre-treatment of D-U937 cells with 40 mg/mL of extract prepared from the Sichompu and Rongrien cultivars of rambutan minimally, but significantly (8% and 11%, respectively) decreased LPS-induced secretion of TNF-α (Figure 5A), but did not affect the concentration of IL-8 in medium (Figure 5B). Flavonoids had been identified in rambutan pulp (Fila et al., 2012) but the specific compounds exhibiting the weak anti-inflammatory activity remain unknown. To our knowledge, this is the first study assessing anti-inflammatory capacity of rambutan pulp.

The selective inhibitory effect of the extracts on the release of TNF-α and IL-8, but not IL-6 and MCP-1 in the present study is in agreement with a previous study demonstrating that Grindelia robusta extract inhibited dose-dependently the secretion of IL-6, RANTES, MCP-1 and, to a lesser extent, PGE2 and TNF-α (La et al., 2010).

A link between antioxidant and anti-inflammatory properties of some fruit extracts consumption and decrease severity of inflammatory-related diseases such as cardiovascular disease was demonstrated in ApoE deficient mice (Xie et al., 2011) and in human (Buscemi et al., 2012). ApoE(-/-) mice were fed AIN-93G diet or AIN-93G supplemented with 5% freeze-dried açai juice powder (AJ) for 20 weeks. The mean lesion areas in the aorta for apoE(-/-) mice fed AJ were 58% less (P<0.001) compared to that of control. Biomarkers of lipid peroxidation, including F (2)-isoprostanes and isomers of hydroxyoctadecadienoic acids and hydroxyeicosatetraenoic acids were significantly lower in serum and in liver of AJ fed mice. Expression of the glutathione peroxidase and glutathione reductase was significantly up-regulated in the aorta from AJ fed mice. Serum levels, gene expression and protein levels of TNF-α and IL-6 in the resident macrophages with or without LPS stimulation were lower in mice fed AJ. Another study in human showed that nondiabetic subjects with increased cardiovascular risk (aged 27–56 y) significantly improved endothelial function and was normalized after drink 500 mL of red orange juice (enriched with phenolic compounds) per day for 7 day. Serum high-sensitivity C-reactive protein, IL-6 and TNF-α in red orange juice consumption group significantly decreased when compared with those of placebo group (Buscemi et al., 2012).

**Conclusion**

The present results suggest that Durian pulp possesses greater antioxidant and anti-inflammatory activity than pulp from rambutan. Also there was a difference in the activities of the extract from the Monthong cultivar compared to the Chanee cultivar of durian. Similarly, extract from the pulp of the Sichompu cultivar of rambutan had greater antioxidant activity than the Rongrien, although there was no significant difference in anti-inflammatory activity of extracts prepared from these two cultivars. Due to higher antioxidant and anti-inflammatory potency of durian than rambutan, these functional activities of durian pulp warrant confirmation in in vivo.
Acknowledgements

This study was supported by Thailand Research Fund (RDG5420059). Authors thank Professor Dr. Mark Failla at The Ohio State University for his helpful comments during preparation of the manuscript.

References


Khonkarn, R., Okonogi, S., Ampasavate, C. and Anuchapreeda, S. 2010. Investigation of fruit peel extracts as sources for compounds with antioxidant and antiproliferative activities against human cell lines. Food and Chemical Toxicology 48(8-9): 2122-2129.


Periasamy, R., Kalal, I. G., Krishnaswamy, R. and Viswanadha, V. 2014. Quercetin protects human peripheral blood mononuclear cells from OTA-induced oxidative stress, genotoxicity, and inflammation. Environmental Toxicology: n/a-n/a.


