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Consumption of anthocyanin-rich Queen Garnet plum juice reduces platelet activation related thrombogenesis in healthy volunteers

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ABSTRACT

The anti-thrombotic properties of an anthocyanin-rich Queen Garnet plum juice (QGPJ) and anthocyanin-free prune juice (PJ) were studied in this randomised, double-blind, crossover trial. Twenty-one healthy subjects (M = 10, F = 11) consumed QGPJ, PJ or placebo, 200 mL/day for 28-days followed by a 2-week wash-out period. Only QGPJ supplementation inhibited platelet aggregation induced by ADP (<5%, $P = 0.02$), collagen (<2.7%, $P < 0.001$) and arachidonic acid (<4%, $P < 0.001$); reduced platelet activation-dependent surface-marker P-selectin expression of activated de-granulated platelets (<17.2%, $P = 0.04$); prolonged activated-partial thromboplastin clotting time (>2.1 s, $P = 0.03$); reduced plasma-fibrinogen (<7.5%, $P = 0.02$) and malondialdehyde levels, a plasma biomarker of oxidative stress ($P = 0.016$). PJ supplementation increased plasma hippuric acid content ($P = 0.018$). QGPJ or PJ supplementation did not affect blood cell counts, lipid profile, or inflammation markers. Our findings suggest that QGPJ but not PJ has the potential to significantly attenuate thrombosis by reducing platelet activation/hyper-coagulability and oxidative stress.

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Abbreviations: ADP, adenosine diphosphate; aPTT, activated partial thromboplastin time; BMI, body mass index; C3GE, cyanidin-3-glucoside equivalents; FITC, fluorescein isothiocyanate; GAE, gallic acid equivalents; HDL, high density lipoprotein; HS-CRP, high sensitivity C-reactive protein; LDL, low density lipoprotein; MDA, malondialdehyde; MFI, mean fluorescence intensity; MPV, mean platelet volume; PAR-1, protease activated receptor-1; PAR-4, protease activated receptor-4; PBO, placebo; PJ, prune juice; PPP, platelet poor plasma; PRP, platelet rich plasma; PT, prothrombin time; QGE, quercetin glucoside equivalents; QGPJ, Queen Garnet plum juice; TC, total cholesterol; TE, trolox equivalents; TG, triacylglycerol; TxA_2, thromboxane A_2

Chemical compounds: Cyanidin-3-glucoside (PubChem CID: 441674); cyanidin-3-rutinoside (PubChem CID: 44256715); hippuric acid (PubChem CID: 464); malondialdehyde (PubChem CID: 10964)

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1. Introduction

Hyperactivity or hyper-aggregation of platelets is a precursor to a number of pro-thrombotic disease states such as cardiovascular disease (CVD) (Siddiqui, Kumar, & Dikshit, 2013). In the event of endothelial vessel wall damage, circulating platelets attach and aggregate at the surface of the damaged endothelium. They consequently undergo activation in response to stimuli that triggers activation-dependent platelet surface-receptors such as $P2Y_1$ and $P2Y_{12}$ (ADP receptors), thromboxane A_2 (TxA_2), $\alpha 2_A$ epinephrine, PAR-1 and PAR-4 thrombin, GPVI and $\alpha_2\beta_1$ collagen receptors (Coughlin, 2000; Dorsam & Kunapuli, 2004; Kehrel et al., 1998; Rivera, Lozano, Navarro-Nunez, & Vicente, 2009). Current anti-platelet drugs block such receptors to reduce platelet hyper-activation and help reduce risk of thrombosis in vascular conditions. Though anti-platelet drugs such as aspirin and clopidogrel have been mainstay treatments in reducing thrombotic risks, there have been several reports of resistance and sensitivity (Goh, Churilov, Mitchell, Dowling, & Yan, 2013; Petricevic et al., 2013). Combination therapy using aspirin, clopidogrel and warfarin has also been associated with significant bleeding risk (Khurram et al., 2006).

The ability of anthocyanin-rich foods to simultaneously target various platelet activation pathways and potentially attenuate thrombosis is currently receiving significant interest (Alvarez-Suarez et al., 2014; Pojer, Mattivi, Johnson, & Stockley, 2013; Santhakumar, Bulmer, & Singh, 2013). Anthocyanins are a main polyphenol subclass and are some of the most abundant polyphenols in fruits and vegetables with an estimated mean intake in Europe of 65 mg/day (Zamora-Ros et al., 2011). Studies suggesting an ability of anthocyanin-rich foods to attenuate thrombosis include: purple and red grape juice supplements inhibiting platelet aggregation in healthy subjects as well as reducing oxidised LDL and activity of NADPH oxidase in dialysis patients (Castilla et al., 2008; Freedman et al., 2001), red wine preventing the typical postprandial increase in lipid hydroperoxides and cholesterol oxidation products when consumed together with the meal (Natella et al., 2011), and anthocyanins, along with colonic polyphenol metabolites, exhibiting *in vitro* inhibitory properties of platelet activation and aggregation (Rechner & Kroner, 2005).

Queen Garnet is a new anthocyanin-rich Japanese plum (*Prunus salicina*) cultivar that was developed in a Queensland Government breeding program and shown to contain up to 272 mg anthocyanins/100 g fresh weight (Netzel et al., 2012). In a pilot trial with two healthy male subjects, 400 mL Queen Garnet plum juice (QGPJ) containing 1.12 g anthocyanins and 2.66 g total phenolics decreased the urinary excretion of malondialdehyde, a biomarker for lipid peroxidation (McKay et al., 2010), within 24 h (Netzel et al., 2012).

In the present study, the potential of anthocyanin-rich QGPJ was compared with anthocyanin-free commercial prune juice (PJ), in inhibiting platelet hyper-activity mediated by various platelet activation pathways. The hypothesis was that anthocyanin-rich QGPJ may ameliorate platelet activation related thrombogenesis and maintain haemostatic function by: (1) reducing platelet aggregation and activation through blocking/inhibiting various platelet activation pathways; (2) prolonging clotting time and reducing fibrinogen concentration; and (3) exhibiting favourable effects on lipid profile and inflammation. Furthermore, the concentrations of hippuric acid, a colon microbial/liver derived metabolite of dietary polyphenols and potential biomarker for total polyphenol intake (DuPont, Bennett, Mellon, & Williamson, 2002), and malondialdehyde were measured using HPLC in urine and plasma of the subjects pre and post treatment to confirm uptake of polyphenols/metabolites and measure their impact on plasma oxidation.

2. Materials and methods

2.1. Study subjects and experimental design

This trial was approved by the Griffith University Human Research Ethics Committee, Griffith University, Queensland, Australia (GU Protocol No. MSC/02/12/HREC) and registered at the Australian New Zealand Clinical trials registry (ACTRN12612000674831).

Twenty-one healthy volunteers (11 men and 10 women) were recruited from the local community and provided informed, written consent prior to participation in the study. Participants were screened by means of questionnaires to be apparently healthy, non-smokers, with no history of metabolic or cardiovascular diseases, and not consuming daily health/energy supplements, anti-platelet or anti-inflammatory medications during the duration of the study. Volunteers on high antioxidant diets were screened using dietary antioxidant questionnaires and were excluded from the study. Initial screening using baseline full blood counts (FBC), biochemical profile, BMI and blood pressure determined that volunteers were within normal reference ranges (Table 1) as established by the Royal College of Pathologists of Australasia (RCPA) (The Royal College of Pathologists of Australasia, 2004).

A randomised, double blind, placebo-controlled, cross-over trial was performed (Fig. 1). After initial screening

Table 1 – Baseline parameters of subjects under study.

Parameters	Male ($n = 10$)	Female ($n = 10$)
Age (years)	32.9 ± 11.8	34.2 ± 10.8
BMI (kg/m^2)	21.2 ± 2.6	19.6 ± 1.5
BP (mm Hg)	127 ± 9	121 ± 8
	76 ± 6	74 ± 7
Haemoglobin (g/L)	138.3 ± 5.4	129.9 ± 9.8
Haematocrit (%)	40.6 ± 1	38.4 ± 2
RBC ($\times 10^{12}$/L)	4.9 ± 0.1	4.6 ± 0.3
WBC ($\times 10^9$/L)	6.0 ± 1.7	7.0 ± 2.0
Platelets ($\times 10^9$/L)	227 ± 41	262 ± 55
MPV (fL)	8.1 ± 1.0	8.0 ± 0.7
TC (mmol/L)	4.42 ± 0.86	4.82 ± 0.86
HDL (mmol/L)	1.40 ± 0.35	1.64 ± 0.42
TG (mmol/L)	0.85 ± 0.30	1.05 ± 0.45
LDL (mmol/L)	2.63 ± 0.83	2.70 ± 0.84
Glucose (mmol/L)	5.15 ± 0.41	4.88 ± 0.37

Values are represented as mean ± SD.
Abbreviations: BP, blood pressure; RBC, red blood cell; WBC, white blood cell; MPV, mean platelet volume; TC, total cholesterol HDL, high density lipoprotein; TG, triacylglycerol. Baseline data between men and women were not significantly different ($P > 0.05$).