Oil palm phenolics as a bioactive ingredient in promoting cardiovascular health

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Abstract
Some plant-based bioactives are known to possess multiple health benefits, and polyphenols represent the largest group of these. The presence of multifunctional (antioxidant, anti-lipid peroxidative and metal chelating) properties within the same molecule enables polyphenols to confer pleiotropism, akin to several therapeutic agents. With such emerging positive scientific evidence, novel polyphenols are actively being pursued by the food and supplement industries as a natural, safe and cost-effective means of delivering health benefits to consumers. Several naturally occurring polyphenol compounds (e.g. tea, grape, wine) have been shown to afford cardiovascular protection through their actions on the vasculature.

The palm oil industry is a global provider of several natural antioxidants. Palm carotenes rich in α-carotenes, and palm vitamin E enriched in tocotrienols reside in the fruit mesocarp, and are recovered from extracted crude palm oil. In addition, a novel water-soluble antioxidant complex enriched in polyphenols (oil palm phenolics; OPP) has been isolated from the large aqueous biomass generated during the milling process. In the present study we evaluated the potential cardiovascular outcomes of OPP via a series of in vitro and in vivo studies following both acute administration and long-term feeding in rats.

Keywords: bioactives, oil palm polyphenolics (OPP), cardiovascular health; blood pressure, nitric oxide, rat

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OPP dose-dependently relaxed isolated blood vessel preparations, and lowered blood pressure (BP) in the nitric oxide deficient model of hypertension as well as in the spontaneously hypertensive rat following acute administration. OPP afforded protection against cardiac arrhythmia following induction of myocardial ischemia by coronary artery ligation in a whole animal model of sudden cardiac death. The results thus implicate OPP as a potential bioactive ingredient effective in promoting cardiovascular health.

1. Introduction

The term “cardiovascular disease” (CVD) includes all diseases and conditions of the heart and blood vessels. CVD remains a major health problem in many developed and developing economies across the globe. CVD deaths resulting from raised blood pressure are, on a worldwide basis, the highest for any risk factor (13%) followed by tobacco smoking (9%), physical inactivity (6%), obesity (6%), diabetes (5%) and cholesterol (4%). The World Health Organisation (WHO) also estimates that globally, at present approximately one billion people have high blood pressure (BP) (hypertension), a figure that is predicted to rise to 1.56 billion people by 2025 (WHO 2013). Approximately 8 million annual deaths worldwide are due to high blood pressure, and 1.5 million of these are in the region of South-East Asia.

Hypertension is a major risk for CVD and cerebrovascular diseases\(^1\),\(^2\). The state of health of the blood vessel network plays a pivotal role in the control of BP, as the tone of smaller blood vessels (arterioles) is a key determinant of peripheral resistance and therefore in the flow of blood. Current evidence also suggests that oxidative stress (altered redox signalling), which results in an excessive generation of reactive oxygen species (ROS), plays a vital part in the development of CVD including hypertension, atherosclerosis, diabetes, cardiac hypertrophy, heart failure, ischemia-reperfusion injury and stroke.\(^3\),\(^4\)

Although hypertension and other cardiovascular abnormalities are controllable by therapeutic means, diet-based strategies to lower BP and promote overall cardiovascular health are becoming increasingly popular, as they may act as a safer and more cost-effective alternative when compared to conventional drug therapy.\(^5\),\(^6\) Indeed, the global demand for natural antioxidants and related bioactives is rising with the emerging evidence that such components play vital roles promoting health and well being.\(^7\),\(^8\),\(^9\)

Natural antioxidants are primarily plant phenolic compounds and include tocoferol and tocotrienols, flavonoids and non-flavonoids such as phenolic acids (eg. gallic, chlorogenic and ferulic acids), and stilbenes (resveratrol). Compared to the benefits of vitamin antioxidants (vitamins E and C) and carotenoids, which have been known for some time, the potential health attributes of dietary polyphenols have only been identified relatively recently.\(^9\),\(^10\),\(^11\),\(^12\)

The reported cardiovascular protective actions of polyphenols include regulation of inflammation, inhibition of platelet aggregation, vascular protection, lowering of blood pressure, protection against cardiac arrhythmia and ischemic damage, inhibition of growth and proliferation of vascular smooth muscle, inhibition of oxidation of low-density lipoproteins and reduction in atherosclerotic lesions.\(^7\),\(^12\) Whilst the scavenging of excessive free radicals by antioxidants is regarded as being central to their mode of action, more recent data also indicates that plant polyphenols possess a range of health benefits which extend beyond their antioxidant/radical scavenging activity.

The continuing increase in demand for natural antioxidants with potential health benefits has also triggered the food and allied industry sectors to seek new sources of raw materials, as well as to add value to existing products and manufacturing processes. In particular, palm oil industry is a global provider of several natural antioxidants as the fruit (Elaeis guineensis) from which palm oil is extracted boasts a rich mix of deep colours (maroon,
red, orange and yellow) which are indicative of an abundance of polyphenolics and carotenes. Indeed, palm carotenes and palm vitamin E, enriched in tocotrienols, reside in the mesocarp, and are recovered from extracted crude palm oil. The vegetation liquor that originates in the milling process and extraction of palm oil has recently been identified as a rich source of water soluble polyphenolic compounds, in contrast to such lipid-soluble antioxidants present in the oil. In the present study we assessed the potential cardiovascular benefits of OPP by developing a robust bioactive discovery process spanning from the identification of target mechanisms and in vitro screening assays to pre-clinical evaluation in whole animal models.

2. Materials and Methods

Animals
The use of animals in the present studies was approved by the CSIRO Food & Nutritional Sciences Animal Experimentation Ethics Committee. All experimental procedures including the care, handling and maintenance of the experimental animals were performed according to the NHMRC guidelines for the use and care of animals for experimental purposes.

2.1. Blood Pressure and vascular studies

2.1.1. SHR model
Spontaneously hypertensive rats (SHR) of 4 week old were maintained on a standard laboratory rat diet formulated to contain low n-3 polyunsaturated fatty acids (Glen Forrest Stock feeders, Glen Forrest, W.A., Australia). OPP was provided as a drink (1500 and 3000 GAE; 30ml/rat/day), and treatments continued for 20 weeks. BP was monitored fortnightly in conscious rats by a standard tail-cuff procedure.

2.1.2. L-NAME model
12 week old Sprague-Dawley rats were provided with OPP (as a drink) at two different strengths (1500 and 3000mg/l GAE; 30ml/rat/day) for four weeks prior to challenge with L-NAME (NG-nitro-L-arginine methyl ester; 15 mg/kg in drinking water), and treatments continued for a further two months thereafter. The intakes were adjusted every two days to allow for gain in bodyweight.

2.1.3. Acute model
13 week old male SHR were familiarized with the procedure for tail cuff BP measurements and baseline readings were established. A single oral dose of each test compound (fractionated OPP) was given via pipette placed at the back of the tongue, and BP readings were taken from treated animals at 0, 3, 6, 9, 24 and 48 hours after administration. All treatments were conducted as pairs. At the completion of the 48 hour BP cycle, animals were returned to the colony and rested for 1 week. Control animals received saline, and enalapril (4mg/kg) served as the positive control. A second round of blood pressure measurements was conducted in the same animal group one week later.

2.1.4. Vascular function
Potential of OPP fractions to lower vascular resistance was also assessed using in vitro vascular preparations (aortic rings and perfused mesenteric vascular bed) as previously reported.

2.1.5. Cardiac arrhythmia
Male WKY rats (Animal Resource Centre, Western Australia) of 12 weeks of age were randomly divided into 3 experimental groups (n=25 per group). Three groups were maintained on a pro-arrhythmic diet low in long chain n-3 polyunsaturated fatty acids and supplemented (5%w/w) with lard (total fat 10% w/w). One group acted as the control (water), while the other phenolics treated group was provided with OPP (1500 mg/l GAE) as a liquid at a rate of 30-35 ml/rat/day. Another group of rats was fed the above diet containing 10% (w/w) total fat, but the saturated fat component (lard 5% w/w) was replaced with fish oil (5% w/w). High-DHA fish oil was a gift from Nu-Mega Ingredients Australia. This preparation contained over 30% long chain n-3PUFAs as DHA and EPA (27% DHA and 6% EPA). The treatments were administered for a period of 4 months. Bodyweights were recorded fortnightly, and animals were monitored routinely for overall health and well-being.

Myocardial ischemia was induced by temporary occlusion of the LAD (left anterior descending coronary artery), as described in detail in previous publications from this laboratory. In brief, rats were anaesthetised (sodium pentobarbitone 60mg/ml stock; 1.0 ml/kg, ip) and intubated. After tracheotomy, animals were ventilated with room air using a
rodent ventilator. The femoral artery and vein were cannulated to monitor blood pressure (BP) and for the administration of saline/drugs/test compounds as required. After left thoracotomy the heart was exteriorised from the thoracic cavity and using an atraumatic needle (6/0 braided silk), a ligature was placed just beneath and around the LAD coronary artery. The heart was returned to the chest cavity and the animals allowed to stabilise (5 min) prior to induction of regional ischemia (occlusion phase) by tightening the ligature. The LAD coronary artery occlusion was maintained for 30 min. to quantify ischemia-induced arrhythmia. At the completion of the experiment, the rats were exsanguinated, hearts excised and perfused with dye (Evans blue, 0.1 ml) to determine the zone-at-risk.

2.2. Statistics
Data were expressed as mean ± SEM. The incidences of VT and VF were compared using the Chi-square test. Differences in the number of VPB, and the duration of VT and VF were subjected to one way ANOVA followed by the Tukey's post hoc test or compared using the Students t-test as relevant. A p value of <0.05 was considered significant. The statistical analyses were performed using the Graph Pad–In Stat (version 4.0) computer software program.

3. Results
3.1. Blood Pressure
Figure 1 shows the development of BP in the SHR over time compared to the normotensive WKY control animals. In contrast to the WKY rats which showed a considerable lower BP level (<150 mmHg), the basal BP level of SHR was increased over time and reached stable level when the animals were 3 months old with a basal BP of >210 mmHg. The long term feeding of OPP (1500 mg/l GAE) did not influence blood pressure in this model, which is a genetic model of hypertension.

![Figure 1: Effect of dietary OPP on blood pressure development in adult spontaneously hypertensive rats.](image)
Figure 2: Effect of OPP in the nitric-oxide deficient model of hypertension

Note: Animals were treated with L-NAME (N\(^\text{N}\)–nitro-L-arginine methyl ester, 15mg/kg for 8 weeks in the presence and absence of two different strengths of OPP (1500 and 3000 mg/L GAE). Final BP values are shown. In contrast, OPP feeding led to a significant lowering of blood pressure in the nitric oxide deficient model of hypertension (L-NAME model). Inclusion of L-NAME (15mg/Kg) in the drinking water gradually increased the BP during the treatment period (data not shown). The final BP values (mmHg) at the conclusion of the experiment were - untreated control 139±1.6; L-NAME control 176±2.5; OPP-1500 mg/l GAE 160±2.1** OPP-3000 mg/l GAE 156±4.4*** (**p<0.01, ***p<0.001, n=12 per group). However, the OPP treatment did not result in a complete normalisation of BP to the basal level of the untreated control group (139±2.8 mmHg).

Figure 3: Effect of fractionated OPP on relaxation of aortic rings

Note: Isolated segments (3 mm) of thoracic aorta were mounted under isometric conditions in organ bath chambers. The aortic rings tissues were equilibrated for 60 minutes before contracting with KCl (20 mmol/L) to test tissue viability. The change in tension was monitored by a computer based data acquisition system. The rings were pre-contracted with half-maximal (EC50) dose of noradrenalin prior to the addition of test samples directly to the bath. F1-F4 refers to the HPLC fractionated samples derived from native OPP (Sambanthamurthi et al., unpublished).
Figures 4 (a&b) shows the effect of enalapril and two purified fractions of native OPP (Sambanthamurthi et al., unpublished) on BP in the SHR. The two OPP fractions were selected based on their ability to cause relaxation in the isolated vascular preparations (Figure 3).

The test compounds were administered as a single oral dose and effects monitored over a 48 hour period. Enalapril (4mg/kg) reduced BP in the 3-9 hr time window (26-43mmHg) with the greatest reduction occurring at the 9 hr measurement point. The BP values returned to baseline by the 24 hr point. Similarly, the OPP fractions also caused considerable reduction in mean BP (7-27mmHg) during the same time course. Both OPP fractions exerted their highest reduction at 6 hr post administration compared to the effects of enalapril which peaked at 9hr after administration. The extent as well as the time profile BP reduction were similar between the two OPP fractions tested (Figure 4b).
3.2. Cardiac arrhythmia:
The control animals treated with L-NAME (in the BP study) and the group that received the higher dose of OPP (3000 mg/l GAE) along with L-NAME were subjected to coronary artery ligation and the key findings - duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) - are presented in Figure 5.

Compared to the L-NAME control rats, the co-administration of OPP (3000 mg/l GAE) resulted in considerable cardio-protection, as evidenced by the significant reduction (P<0.01 or better) in the duration of VT (control 35.7±7.8 sec.; OPP 2.3±1.0 sec., n=9); there were no episodes of VF in the OPP supplemented group compared to the control rats treated with L-NAME which displayed VF with a mean duration of 28.2±15.6 seconds.

Figure 5: The duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) in rats treated with NG-nitro-L-arginine methyl ester (L-NAME) for 12 weeks.
The potential effect of long-term dietary supplementation with OPP against a pro-arrhythmic dietary background was investigated in the normotensive (WKY) rats. In this experiment, animals were fed a diet rich in saturated fats (10% w/w, derived from lard) in the presence and absence of OPP. Table 1 summarises the cardiac arrhythmia parameters at the end of feeding period (20 weeks).

The % incidence of VT was similar across the 3 groups, while the VF incidences were reduced following OPP supplementation or in the presence of longchain omega-3 fatty acids in the diet (FO group). Compared to the high incidence of VF (90%) in the control group, only half of the rats (13/25) in the OPP group developed VF, and in the FO group only 7 animals out of 20 suffered VF episode. Similarly, total mortality was lowest in the FO group. The OPP feeding was also associated with lower mortality (20%) (5/23) compared to the control rats (9/23). Final bodyweight were similar across groups and no difference was observed in the area of myocardium rendered ischemic (zone-at-risk) by ligation of the coronary artery (Table 1).

4. Discussion
The present experiments involved both acute and long-term feeding studies (up to 20 weeks duration) of OPP in different strains of rats (Sprague Dawley, WKY and SHR), and there were no apparent ill effects of polyphenol supplementation on growth and development, as the bodyweights were similar across experimental groups. The elevated blood pressure in the adult SHR was not influenced by supplementation with dietary polyphenols. In contrast, OPP was effective in reducing the rise in BP in the L-NAME model of hypertension. This latter model, which reflects a deficiency of endogenous nitric oxide (NO), was positively influenced by OPP, suggesting that palm phenolics may have been effective in stimulating the production and/or release of NO.

Our previous studies using isolated vascular preparations have in fact indicated that the vascular relaxation properties of OPP are likely to be mediated via NO-related mechanism(s). The fact that long-term low dose feeding of OPP in the SHR did not reduce BP, but a bolus administration of OPP (250mg/kg) caused reduction of blood pressure in the SHR, lends further support to direct vasodilatory actions of OPP. The most likely mechanism for reduction in BP in this latter setting (acute administration, Figure 4) is a change in the total peripheral resistance due to a sudden surge in endogenous NO triggered by a higher circulating dose of OPP.

The extent of reduction in BP (up to 24-27mmHg) we observed following single dose administration of purified OPP (250mg/kg) is similar to those reported recently for this acute blood pressure model of SHR, for example, boysenberry seed polyphenols (200mg/kg resulting in 17mmHg reduction), cocoa polyphenols extract (26-39mmHg) and cocoa powder. The latter investigators used a dose range of 50-600mg/kg and found change in BP of 34-58 in the lower end of the dose range but the highest dose tested (600mg/kg) had a much lower effect (20mmHg). Only a single oral dose was tested in the present study, and further work is necessary to establish the optimal dose-range of OPP that leads to the most favourable outcomes.

Evidence for anti-hypertensive actions of OPP are also observed in the NO-deficient L-NAME-model, where OPP resulted in 16-20 mmHg reduction in BP at the end of 8 week feeding period. These findings are also in agreement with several previous studies which reported 8-40 mmHg reduction in BP following long-term dietary feeding of different polyphenols.
Table 1: Cardiac arrhythmia parameters of rats following long-term dietary supplementation with OPP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>OPP</th>
<th>FO</th>
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<tbody>
<tr>
<td>VT (% incidence)</td>
<td>96 (22/23)</td>
<td>88 (22/25)</td>
<td>75 (15/20)</td>
</tr>
<tr>
<td>VF (% incidence)</td>
<td>91 (21/23)</td>
<td>52 (13/25)*</td>
<td>35 (7/20)*</td>
</tr>
<tr>
<td>VF duration (sec)</td>
<td>99.8 ± 21.18</td>
<td>47.5 ± 15.48</td>
<td>28.8 ± 11.5</td>
</tr>
<tr>
<td>% Total mortality</td>
<td>39 (9/23)</td>
<td>20 (5/25)</td>
<td>5 (1/20)*</td>
</tr>
<tr>
<td>% zone-at risk</td>
<td>49.6 ± 0.7</td>
<td>48.6 ± 0.8</td>
<td>48.8 ± 0.9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>398 ± 6</td>
<td>385 ± 6</td>
<td>411 ± 6</td>
</tr>
</tbody>
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Note: Rats were maintained on different dietary supplements for 20 weeks prior to induction of cardiac arrhythmia by coronary artery ligation. OPP was provided via drinking water. Data presented are the Mean±SEM. Numbers within parantheses refer to the number of animals. Asterisk denotes significance p<0.05 or better compared to the control group (p<0.05, or better). VT, ventricular tachycardia; VF, ventricular fibrillation.

The L-NAME treated rats also showed increased vulnerability to develop abnormalities in cardiac rhythm following myocardial ischemia brought upon by coronary artery ligation (Figure 5). OPP feeding was associated with considerable cardio-protection in this model. In rats fed a pro-arrhythmic diet for 20 weeks, OPP was associated with a reduction in VF episodes and lower %mortality rate compared to the control rats (Table 1).

The vascular relaxation and cardioprotective actions of OPP\textsuperscript{13,27} were re-confirmed in the present study, which also demonstrated that further fractionation of native OPP leads to enrichment of bioactivity. In fact, there was good agreement of the in vitro observations (Figure 3) and outcomes in the whole animal model (Figure 4).

Taken collectively, present findings suggest OPP favourably influence several parameters of cardiovascular patho-physiology and implicate OPP as a potential bioactive ingredient. Whilst the exact underlying mechanisms are not clear at this stage, evidence to date implies modulation of vascular endothelial function as the principle basis for the apparent cardioprotective qualities of OPP. In this regard, several polyphenols preparations including grape, wine, cocoa and green tea have also been shown to modulate vascular endothelial function and signalling mechanisms\textsuperscript{9,12,28} in addition their ability to reduce oxidative burden by modulating the expression of antioxidant and pro-oxidant enzymes.

References

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