

## Review Article

# Health benefits of a natural carotenoid rich oil: a proposed mechanism of protection against ischaemia/reperfusion injury

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Numerous studies have reported the protective properties of carotenoid supplementation against skin and eye associated diseases. However, conflicting data concerning the efficacy of β-carotene in the pathogenesis of cancers and cardiovascular disease exist. It has been shown that β-carotene is an effective antioxidant on its own or in combination with other antioxidants. Red palm oil (RPO) is a potent anti-oxidant rich oil which consists of carotenoids, tocopherols, tocotrienols and lycopenes as well as lipid fractions such as squalene, saturated and unsaturated fatty acids (which maximize absorption of these anti-oxidants) and Co-enzyme Q<sub>10</sub>. α and β-carotene account for more than 90% of the total carotene in RPO. It is known that ischaemia/reperfusion-induced injury causes an imbalance in oxygen supply which can lead to oxidative stress in the heart. It has been shown that the mitogen-activated protein kinases (MAPKs), PKB/Akt and the NO-cGMP all play vital roles in ischaemia/reperfusion injury in the heart. Therefore, our review mainly focuses on the signaling pathways involved in functional recovery induced by a natural carotenoid oil after ischaemia/reperfusion injury.

**Key Words:** Anti-oxidant rich oil, carotenoid, tocotrienol, tocopherol, red palm oil, oxidative stress, ischaemia/reperfusion

## INTRODUCTION

Correct eating habits and a healthy lifestyle has become an important issue in the existence of mankind. Consequently, the use of supplements, natural and chemically produced, has become a topical discussion in many nutritional circles. The fact that mankind ignore the importance of dietary fruit and vegetables are exacerbated by the absence of a suitable substitute for these essential components of our diet. This paper focuses on the beneficial effects of consuming a carotenoid rich oil (CRO) to supplement daily nutritional intakes and propose some signalling mechanisms involved CRO-induced protection.

## BENEFICIAL EFFECTS OF CAROTENOID

Two major forms of vitamin A are present in the diet: retinyl esters from animal sources and carotenoid from plants.<sup>1</sup> There are more than 600 carotenoid in nature of which 6 can be measured in blood.<sup>2</sup> Carotenoid are fat soluble pigments which are found in fruits and vegetables.

Carotenoids cannot be synthesised in animals or humans and therefore needs to be part of the dietary intake. α and β-carotene, and lycopenes are the major carotenoids which are mainly composed of carbon and hydrogen atoms. In humans and animals carotenoids play an important role in

protection against photooxidative processes<sup>3</sup> by acting as oxygen and peroxy radical scavengers. Their synergistic action with other anti-oxidants makes them an even more potent compound.<sup>4</sup>

More than 70% of the vitamin A intake in Third World countries comes from fruit and vegetables in the form of carotenoids. Several studies have shown that less plant-derived carotenoids is absorbed, cleaved, reduced and finally available as retinyl esters.<sup>5</sup> Therefore, bioequivalence is less than predicted and factors such as food preparation, matrix properties, co-ingestion of fat, diseases of the gastrointestinal tract and malnutrition may be contributors.<sup>6</sup>

Carotenoids have a wide range of protective properties against disease and aging and can act as a modulator for cellular processes and function. Several studies have shown

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that carotenoids can protect against cancer<sup>7,8</sup> and cardiovascular disease.<sup>9,10</sup> Furthermore, dietary carotenoids can protect the skin against sun exposure,<sup>11,12</sup> whilst anti-oxidant and cellular signalling effects have been shown in a number of studies.<sup>13,14,15</sup>

### SYNERGISTIC EFFECT OF ANTI-OXIDANTS

It has been suggested that different individual compounds with variable anti-oxidant activity may provide additional protection against oxidative stress when ingested simultaneously.<sup>16</sup> A combination of lipophilic anti-oxidants resulted in an inhibition of lipid peroxidation significantly greater than the sum of the individual effects.<sup>17</sup> This may suggest that a cocktail of antioxidants may have a far more profound anti-oxidative effect due to the synergistic action of the individual compounds. It is also significant to note that lycopene or lutein was always present when this phenomenon was noted.<sup>16</sup> These results thus suggest that a combination of carotenoids and vitamin E in the presence of lycopene in a natural food supplement might provide the ultimate dietary supplement to fight disease associated with oxidative stress.

### RED PALM OIL: A CAROTENOID RICH ANTI-OXIDANT OIL

In refined edible red palm oil (RPO) as much as 80% of the carotene and vitamin E originally present in crude palm oil is retained through a novel process of pre-treatment, de-acidification and de-odorization using molecular distillation. The oil contains 500 ppm carotene of which 90% is present as  $\alpha$ -carotene (37%) and  $\beta$ -carotene (47%). Lycopene represents 1.5% of the carotenoids and *cis*- $\alpha$ -carotene 6.9% with all the other minor carotenoids making up the difference. The vitamin E content is about 800 ppm, 70% of it is in the form of tocotrienols (mainly  $\alpha$ -,  $\beta$ -,  $\gamma$ -tocotrienols) and only 30% as tocopherols. Red palm oil contains the highest concentration of tocotrienols compared with other vegetables or plants and Serbinova et al.<sup>29</sup> showed that tocotrienols can be 40-60 times more potent as anti-oxidant than tocopherols. Other minor components present in this oil are ubiquinones and phytosterols.<sup>18</sup> Coenzyme Q<sub>10</sub> is the most common ubiquinone in commercial red palm oil at levels of 18-15 ppm.<sup>19</sup> Red palm oil is therefore a natural carotenoid rich oil that has the potential to act as a very potent anti-oxidant oil.

### PROTECTION OF RED PALM OIL AGAINST OXIDATIVE STRESS

Esterhuyse et al. showed that red palm oil protected the isolated perfused rat heart against ischaemia/reperfusion injury.<sup>20,21</sup> They used a model where red palm oil was added to the diet of rats for 6 weeks at a daily dosage of 0.58 mg/kg weight per day. After this feeding period, hearts were excised and mounted on a perfusion apparatus. The hearts were subsequently exposed to ischaemia for 30 minutes followed by a period of 20 minutes of reperfusion. During the ischaemic period hearts were deprived of oxygen. During reperfusion hearts were re-oxygenated, causing an overload of oxygen and a subsequent oxidative burst, formation of free radicals and a potential to cause severe injury and damage to myocardial cells.<sup>20</sup> Esterhuyse et al. found that red palm oil supple-

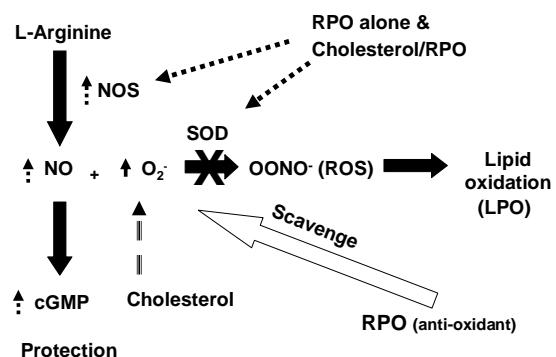
mented hearts had significantly better functional recoveries when compared with control hearts, but could not explain the mechanism of cardio-protection.<sup>20</sup>

### PROTECTIVE MECHANISMS OF RED PALM OIL

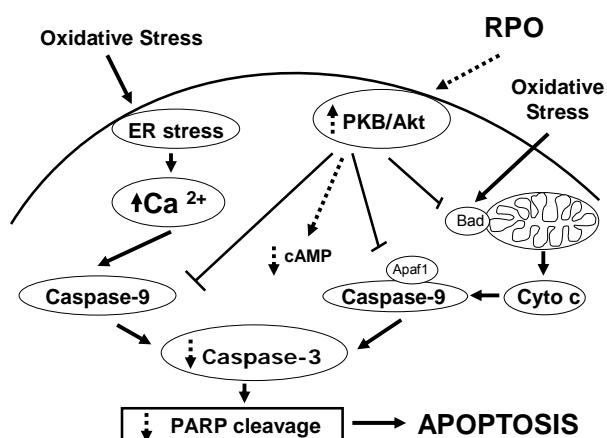
The same group investigated possible mechanisms of protection and published two papers that suggest that two mechanisms may be involved. Esterhuyse et al. investigated the possible role of the nitric oxide (NO) -cGMP pathway, whilst Engelbrecht et al. investigated the role of the PKB/Akt and the MAP kinases pathway.<sup>22,24</sup>

#### Protection via NO-cGMP

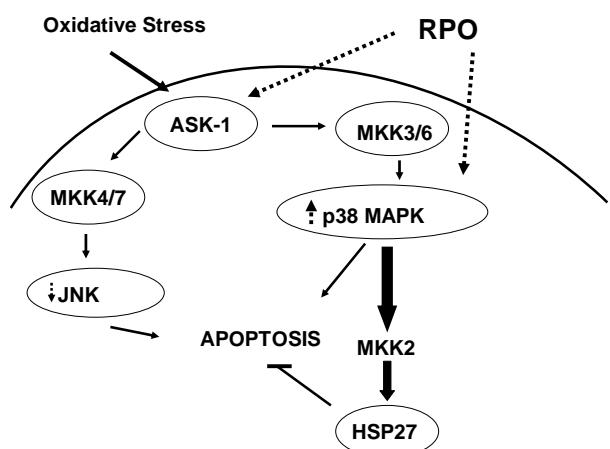
Nitric oxide has been shown to be cardioprotective against the consequences of ischaemia/reperfusion and it has been shown that these protective effects are mediated through cGMP.<sup>24</sup> Esterhuyse et al. showed that ischaemic cGMP levels were significantly increased in RPO treated hearts.<sup>22</sup> Associated with this finding, cardiomyocyte NO production was also significantly increased whilst in cholesterol and RPO supplemented hearts myocardial nitric oxide synthase (NOS) activity also increased. Their results also showed that RPO could not influence the activity of cardiac SOD and cardiac lipid hydroperoxide production. Although Esterhuyse et al did not measure cardiac O<sub>2</sub><sup>-</sup> production, Girics et al. has shown that cholesterol increases the production of O<sub>2</sub>.<sup>25</sup> Increased NO and O<sub>2</sub><sup>-</sup> would favour a reaction in which ONOO<sup>-</sup> is produced and LPO increases. However, this was not the case since cardiac SOD activity was unchanged. Instead the reaction followed the pathway to cGMP production. They therefore speculated that the high anti-oxidant content (carotenoids, tocotrienols and tocopherols) in the RPO, may have scavenged the O<sub>2</sub><sup>-</sup>, increasing the bioavailability of NO to stimulate guanylyl cyclase, and increase myocardial ischaemic cGMP levels. It is also interesting to note that these changes in the NO-cGMP pathway took place very early in ischaemia. No changes could be detected in reperfusion. Figure 1 shows the possible effect of RPO on the NO-cGMP pathway early in ischaemia.



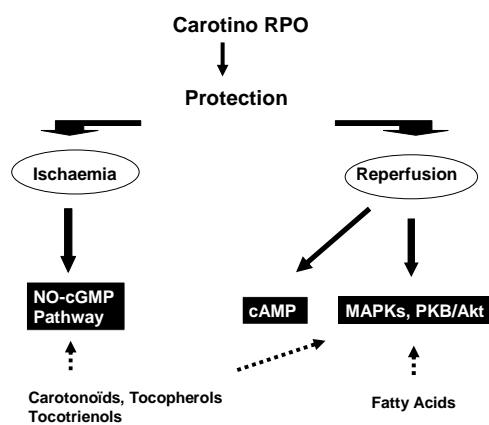
**Figure 1.** The NO-cGMP Pathway. Solid arrows represent the normal occurring pathway. Dotted arrows show the effect of red palm oil supplementation on the levels and production of substrates in the pathway. Intermittent line shows the effect of cholesterol feeding on superoxide. Open arrow show the possible anti-oxidant effect of red palm oil. NOS= Nitric Oxide Synthase; NO= Nitric Oxide; O<sub>2</sub><sup>-</sup>= Superoxide; SOD= Superoxide Dismutase; ONOO<sup>-</sup>= peroxinitrate; RPO=red palm oil.



**Figure 2.** Oxidative stress causes a cascade of events that would eventually induce apoptosis (black arrows). The effects of red palm oil supplementation are shown with the dotted lines. Solid lines indicate where the effects of red palm oil supplementation could have had its effect. Phosphorylation of PKB/Akt could inhibit Caspase 9, which would have a downstream effect to protect against apoptosis.



**Figure 3.** The effect of oxidative stress on the ASK and MAP kinase pathway. The effects of red palm oil supplementation are shown with the dotted lines. Both JNK and p38 phosphorylation was affected with consequent downstream effects that protect against apoptosis. Thick solid lines indicate a possible mechanism for p38 MAPK involvement.



**Figure 4.** The effect of red palm oil supplementation on oxidative stress. Solid arrows show that RPO supplementation had two effects: one in ischaemia and one in reperfusion. Dotted arrows suggest the involvement of certain compounds of red palm oil on specific signalling mechanisms.

### Protection via PKB/Akt and the MAP kinases

It is generally accepted that PKB/Akt promotes survival of the cardiomyocyte and that it protects against ischaemia/reperfusion injury.<sup>26</sup> Several investigators have reported that p38 MAP kinase may also protect against ischaemia and reperfusion injury. Conflicting results on the role of JNK in cardioprotection have been reported. Some believe it is pro-apoptotic while others have shown that it can protect the cardiomyocyte against injury.<sup>27,28</sup> Engelbrecht *et al.* found that RPO caused a significant increase in aortic output recovery compared to control hearts.<sup>23</sup> This was associated with significant increases in phosphorylation of cardioprotective PKB/Akt in the RPO supplemented group. Phosphorylation of pro-apoptotic JNK 54 and JNK 46 was significantly reduced. Simultaneously, RPO attenuated the PARP cleavage, which is a pro-apoptotic modulator. Interesting to these findings was the fact that these significant changes took place during the reperfusion period only. Figure 2 and 3 shows a schematic illustration to explain the effect of RPO on the PKB/Akt, p38, JNK, caspase 3 and PARP cleavage.

### Two pathways involved in the protective effect of RPO

From the results of Esterhuyse *et al.* and Engelbrecht *et al.* it is evident that two pathways may play a role in the protection induced by RPO against ischaemia/reperfusion injury.<sup>22,23</sup> The results of Esterhuyse *et al.* shows that protection may occur during ischaemia and that NO-cGMP may be involved. Engelbrecht *et al.*, on the other hand, argues for a role for the PKB/Akt and the MAP kinases pathways in the cardioprotective effects of RPO during reperfusion. Figure 4 illustrates the possible mechanism of RPO protection.

### CONCLUSION

Current evidence suggests that RPO, as a natural carotenoid rich oil, may protect the heart against an episode of oxidative stress in the isolated rat heart. Several possible mechanisms exist. The evidence also supports the belief that a combination of carotenoids, lycopene, pro-vitamin E and minor components in a natural matrix of oil provide better protection than any individual compound.

### AUTHOR DISCLOSURES

Jacques van Rooyen, Adriaan J Esterhuyse, Anna-Mart Engelbrecht and Eugene F du Toit, no conflicts of interest.

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