

Lipid- and Water-soluble Palm Antioxidants Reduce Development of Atherosclerosis Plaques in Rabbits

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INTRODUCTION

Oxidation of low-density lipoprotein-cholesterol (LDL-C), the major cholesterol-carrying lipoprotein in plasma, is commonly implicated as an initiator of atherosclerosis. Increased LDL-C concentration is a major risk factor for atherosclerosis in humans (Carmena *et al.*, 2004). Oxidised LDL-C is engulfed by macrophages, a type of white blood cell, and this rapidly leads to the formation of atherosclerotic plaques. The sequential steps in the formation of foam cells (fat-laden macrophages) will eventually culminate in their rupture into fatty streaks. Over time, these fatty streaks mature into fatty plaques and accumulate in the arterial wall, reducing the size of the blood vessel lumen. This inhibits blood flow to the heart and brain and eventually blocks the artery, which may result in a heart attack or stroke (Ross, 1993; Tedgui and Mallat, 2006).

The possible association of atherosclerosis with oxidative events has led to the hypothesis that dietary antioxidants may inhibit the development of atherosclerosis and reduce the incidence of coronary artery disease (Xu *et al.*, 1998; Pandey and Rizvi, 2009). Kinsella *et al.* (1993) reported that natural antioxidants, which are the water-soluble antioxidants (like vitamin C and phenolic compounds including flavonoids) and the fat-soluble an-

tioxidants (like vitamin E and carotenoids), are hypothesised to have the potential to intervene in the development of atherosclerosis and cardiovascular disease by modulating oxidation and reduction reactions in disease progression.

The presence of several fat-soluble micronutrients in palm oil such as vitamin E, carotenoids and phytosterols contributes to its numerous nutritious characteristics. The oil palm fruit has been identified as an excellent source of two major phytochemicals, namely, vi-

tamin E (tocopherols and tocotrienols) and carotenoids, both of which are fat-soluble. Oil palm vitamin E has been reported to act as a potent biological antioxidant, protecting against oxidative stress and the atherosclerotic process (Sundram *et al.*, 2003; Mukherjee and Mitra, 2009). Oil palm fruit have also been identified as a rich source of phenolic compounds (Sambanthamurthi *et al.*, 2011a). A water-soluble extract rich in phenolic acids has been successfully recovered from the vegetation liquor generated from the milling of oil palm fruit (Sambanthamurthi *et al.*, 2011a). Based on studies with animal models, these oil palm phenolics (OPPs) have been found to have bioactive properties with potent protective effects against chronic diseases such as cardiovascular disease, diabetes and cancer (Sambanthamurthi *et al.*, 2011b).

Given that both palm vitamin E (VIT E) and oil palm phenolics are individually protective against atherosclerosis development in an

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atherogenic diet-fed rabbit animal model, recently a study (Idris *et al.*, 2014) was carried out to evaluate these oil palm antioxidants in combination with each other in the same animal model of atherosclerosis for potential synergism.

THE STUDY

In the study (Idris *et al.*, 2014), 32 New Zealand White rabbits were divided into four groups of eight and fed an atherogenic diet for 100 days. Group 1, the Control group (CTR), was fed the atherogenic diet, and Group 2, the Vitamin E group (VIT E), was fed the atherogenic diet with added vitamin E (500 mg vitamin E kg⁻¹ diet). Animals in Groups 1 and 2 were provided with distilled water as their drinking fluid. Group 3, the palm phenolics group (OPP), was fed the atherogenic diet with palm phenolics (at a concentration of 1500 mg gallic acid equivalent litre⁻¹ [mg GAE litre⁻¹]) provided as their drinking fluid, while Group 4, the combination of vitamin E and oil palm phenolics group (VIT E+OPP), was fed the atherogenic diet with added vitamin E, and palm phenolics (1500 mg GAE litre⁻¹) was provided as their drinking fluid. The animals had free access to the diets and drinking fluids.

Results from the study (Idris *et al.*, 2014) show that the CTR group had a significantly higher ($p<0.05$) fibrous plaque score ($8.90\pm 5.41\%$) compared with the VIT E ($2.88\pm 2.01\%$), OPP ($1.48\pm 4.45\%$) and VIT E+OPP groups (Figure 1a). Fatty plaques were significantly higher ($p<0.05$) in both the CTR and VIT E groups compared with the OPP and VIT E+OPP groups (Figure 1b). No occurrence of fibrous plaque or

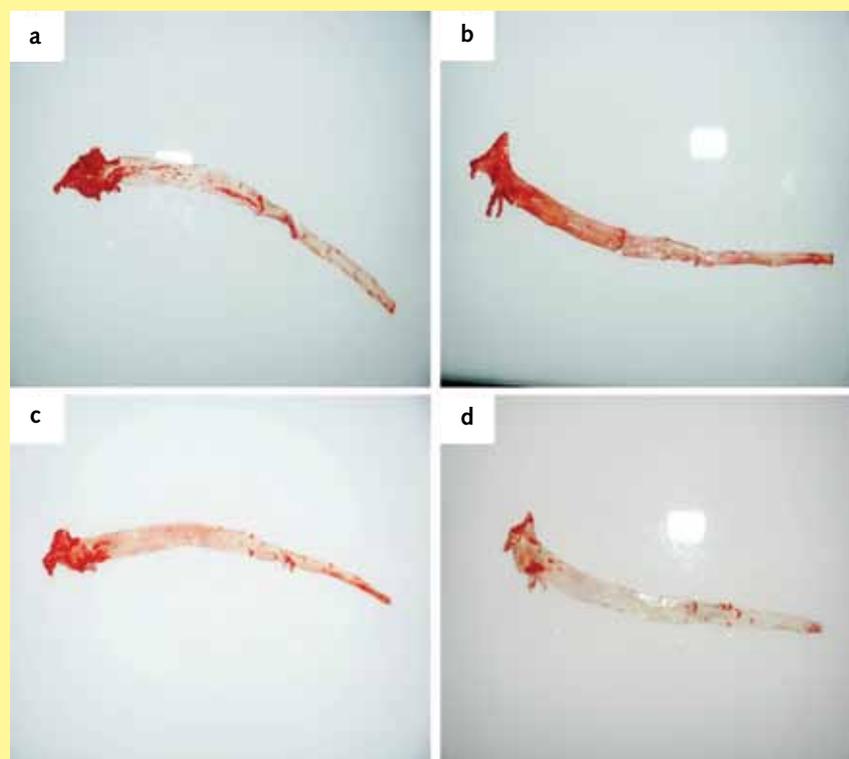
fatty plaque was observed in the VIT E+OPP group. Development of fatty streaks was significantly lower ($p<0.05$) in the OPP and VIT E+OPP groups compared with the CTR and VIT E groups. On the other hand, fatty streak incidence was highest ($23.02\pm 7.75\%$) in the VIT E-fed diet followed by the CTR group (Figure 1c). Lesion-free area (*i.e.* free of fatty streaks, fatty and fibrous plaques) was significantly higher ($p<0.05$) in the VIT E+OPP and OPP groups compared with the VIT E and CTR groups (Figure 1d). This observation suggests protective effects of the OPP and VIT E+OPP treatments against the occurrence of atherosclerosis.

DISCUSSION

Vitamin E (Williams *et al.*, 1992) and phenolic compounds (Yamakoshi *et al.*, 1999) have been documented to inhibit the

development of aortic lesions in rabbits. In the study by Idris *et al.* (2014), synergistic effects were observed between vitamin E and OPP. This observation is consistent with previous findings. A mixture containing both water-soluble and lipid-soluble antioxidants is able to quench free radicals in both aqueous and lipid phases (Chen and Tappel, 1996). Murakami *et al.* (2003) showed that a combination of quercetin or catechins plus α -tocopherol exhibited significantly higher activity than the sum of the individual activities using the liposome oxidation method.

Upaganlawar *et al.* (2009) reported that antioxidants are uniquely different from one another and work synergistically and more effectively when they are used in combination. The observations of Idris *et al.* (2014) are in agreement with those of Stocker



Source: Idris *et al.* (2014).

Figure 1. Photographs of aortic lesions in rabbits fed an atherogenic in different groups (a) CTR, (b) VIT E, (c) OPP and (d) VIT E+OPP.

and O'Halloran (2004) who reported that dealcoholised red or white wine which contains polyphenolic compounds was capable of synergising with vitamin E and decreasing atherosclerosis in apolipoprotein E-deficient (Eo) mice.

One possible mechanism by which oil palm phenolics reduced atherosclerotic lesions is through their anti-inflammatory effects. Cytokines play a significant role in mediating the inflammatory response in atherosclerosis. Atherosclerosis is generally associated with cytokines that promote a Type 1 helper T-cell (Th1) cellular immune response rather than a Type 2 helper T-cell (Th2) humoral immune response. The modulation of the Th1/Th2 axis toward the latter may thus be athero-protective. Leow *et al.* (2013) showed that oil palm phenolics were able to attenuate antigen presentation and processing in the spleens of mice given an atherogenic diet. In addition, oil palm phenolics also modulated the Th1/Th2 axis of the immune system towards the latter, thus suggesting that oil palm phenolics reduced atherosclerosis via their anti-inflammatory actions. This mechanism may thus help explain the changes. In addition to their anti-inflammatory effects, oil palm phenolics also restored the antioxidant capacity of mice fed the atherogenic diet (Leow *et al.*, 2013). Further investigations to discover other possible/probable mechanisms by which oil palm phenolics reduce atherosclerosis are warranted. Animals fed with VIT E + OPP showed a significant reduction ($p < 0.05$) in the development of atherosclerotic lesions compared with animals given either vitamin E alone or oil palm phenolics alone. In a similar study, Xu *et al.* (1998)

reported that reduction of oxidative stress *in vivo* by catechin and vitamin E in hamsters could prevent fatty streak accumulation in the aorta. The effect of oil palm phenolics in inhibiting the formation of fibrous plaques in the present study was evident in both the OPP and VIT E + OPP groups compared with the CTR and VIT E groups, suggesting that oil palm phenolics may potentially inhibit atherosclerosis. Similarly, other phenolic compounds demonstrated this property as well. For example, pomegranate juice (Kaplan *et al.*, 2001) and grape extract (Auger *et al.*, 2004) were found to reduce atherosclerotic lesions when fed respectively to hamsters and mice.

Subsequently, histopathological examination also revealed massive destruction of the tunica intima (the innermost layer of the arteries and veins) in the CTR group indicating endothelial activation (Idris *et al.*, 2014). These fibrous caps were formed due to the proliferation and migration of smooth muscles and connective tissue deposition. The formation of lipid core and foam cells was reduced after administration of vitamin E and oil palm phenolics individually, and especially in combination. These results suggest the great potential of these phytonutrients in mitigating atherosclerosis even when the animals are fed high fat diets.

Thus, the anti-atherogenic effects conferred by oil palm phenolics and vitamin E in the findings of Idris *et al.* (2014) may be explained by the vast mixture of phenolic acids, together with the presence of tocotrienols and tocopherols. These anti-atherogenic mechanisms of oil palm phenolics merit further research attention.

CONCLUSION

The effects of oil palm phenolics (OPPs) either on their own or in combination with vitamin E (VIT E + OPP) in inhibiting atherosclerosis were investigated, and a superior inhibitory effect on the development of atherosclerotic lesions was observed in both the OPP and VIT E + OPP groups compared with the CTR and VIT E groups. These findings suggest a potential area for the application of oil palm vitamin E and oil palm phenolics as antioxidants in modulating cardiovascular risk factors; this merits further research attention. The effects shown by this combination of bioactives present in palm oil also constitute an important factor in the formulation of functional foods and in the choice of a diet which uses the palm oil as a food source.

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