UNIVERSITI TEKNOLOGI MARA

EFFECTS OF PALM OIL DERIVED TOCOTRIENOL RICH FRACTION (TRF) ON CORONARY RISK MARKERS : *IN VITRO* AND *IN VIVO* STUDIES

SUHAILA ABD MUID

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Candidate's Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own, unless otherwise indicated or acknowledge as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

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Name of Candidate	**SUHAILA BT ABD MUID**
Candidate's ID No	2004234628
Programme	Master of Science
Faculty	Medicine
Thesis Title	Effects of Palm Oil Derived Tocotrienol Rich Fraction (TRF) on Coronary Risk Markers: *In Vitro* and *In Vivo* Studies.
Signature of Candidate	
Date	20 JUNE 2008

ABSTRACT

Tocotrienol is one of the vitamin E compounds which have potent anti-oxidant activity leading to reduction in oxidative stress and inflammation. Oxidative stress and inflammation are now emerging as pivotal factors in the pathogenesis of atherosclerosis and coronary artery disease (CAD). However, optimal concentrations of palm oil derived tocotrienol rich fraction (TRF) that may lead to reduction of oxidative stress and inflammation in *in vitro* and *in vivo* studies are still unclear. The objectives of this study were to determine the optimal concentrations of TRF which leads to the highest anti-oxidant activity and reduction of inflammation markers *in vitro* and to study the effects of palm oil derived TRF vitamin E capsules (Palmvitee) contained low dose tocotrienol on oxidative stress in patients with non-familial hypercholesterolaemia (NFH). Anti-oxidant activities were assessed by Ferric thiocyanate (FTC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities and dichlorofluorescein diacetate (DCFHDA) assays. Supernatant of stimulated endothelial cells was measured for the production of inflammatory markers [e-selectin, p-selectin, soluble intercellular cell adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and interleukin-6 (IL-6)]. Sixty-six patients with NFH (38 males, 28 females, age $\pm$ SD age = 46.9 $\pm$ 9.4 years) were recruited and randomised to 3 treatments arm, which were palmvitee 300 mg/day (NFHe), atorvastatin 10 mg/day plus placebo (NFHsp) or atorvastatin 10 mg/day plus palmvitee (NFHse). Fasting serum lipids (FSL), oxidised LDL (ox-LDL), malondialdehyde (MDA) and 8-epi-$PGF_{2\alpha}$ were measured at baseline (BL) and 2 weeks, 12 weeks and 36 weeks post-randomisation. Normocholesterolaemic (NC) subjects were recruited in parallel. In the FTC assay, TRF at concentrations 10 and 100 μg/ml showed the highest percentage (%) of inhibition, 96.4 $\pm$ 0.2% and 96.3 $\pm$ 0.2% respectively. In the DPPH assay, optimal TRF concentration was observed at concentration 62.5 μg/ml with the highest % inhibition (85.2 $\pm$ 0.8%). Lowest % increase of DCF fluorescence production was shown by induced RAW 264.7 cells incubated with 88 μg/ml TRF concentration and it was significantly lower than DCF fluorescence produced by RAW 264.7 cells incubated with inducer (lipopolysaccharides and interferon gamma) alone, 95.0 $\pm$ 1.4% vs. 194 $\pm$ 18.5%, $p<0.05$. The results from the DPPH and DCFHDA assay were consistent with the findings from the FTC method which reported optimal TRF concentration within the range of 10-100 μg/ml. Optimal TRF concentrations leading to maximal inhibition of e-selectin, p-selectin, sICAM-1, sVCAM-1 and IL-6 in stimulated endothelial cells were 0.8, 0.4, 0.8, 3.4 and 0.2 μg/ml, with percentage inhibition of 88.0 $\pm$ 2.0%, 88.0 $\pm$ 0.1%, 55.0 $\pm$ 3.0%, 75.0 $\pm$ 4.0% and 56.0 $\pm$ 12.0%, respectively. NFHe group showed neutral effects on FSL, but reductions in MDA ($p<0.005$), ox-LDL ($p<0.05$) and 8-epi-$PGF_{2\alpha}$ ($p<0.05$) levels at 12 weeks compared to BL. NFHsp and NFHse groups showed similar reductions in TC and LDL at 2 weeks ($p<0.0001$) compared to BL. TG level was also reduced at 2 weeks in both NFHsp ($p<0.01$) and NFHse ($p<0.005$) groups compared to BL. NFHsp and NFHse

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CHAPTER ONE

INTRODUCTION

Oxidative stress plays a major role in the pathogenesis of atherosclerosis (Naurooz et al., 2001). Atherosclerosis is a complex multifactorial disease which is associated with endothelial cell activation, oxidative stress and accumulation of leukocytes in the walls of large arteries, leading to the formation of atherosclerotic lesions (Berliner, 1996). Development of lesions can result in plaque fissures, rupture and thrombus formation, leading to several cardiovascular diseases including coronary artery disease (CAD), myocardial infarction, stroke and heart failure. Cardiovascular disease is a common cause of death in the developed world and is poised to become the most significant health problem worldwide (James, 2004). Malaysia, as a developing country, facing the same problem as well where, CAD has become the leading cause of death. High prevalence of the major coronary risk factors among rural communities in Malaysia has been reported by Nawawi et al., (2002). The coronary risk factors were hypercholesterolaemia, low HDL, obesity, hypertension and diabetes mellitus.

Hypercholesterolaemia is universally accepted as a major risk for atherosclerosis. Elevated plasma low density lipoprotein (LDL) concentration leads to prolonged plasma half-life and increases its susceptibility to free radical attack and oxidation. The increased LDL entry rate into the tunica intima results in a higher steady state concentration of LDL in the intima, which soon developed into arterial lesions (Steinberg, 1997). Endothelial cells, smooth muscle cells, neutrophils and monocytes in the vessel wall have the potential to oxidatively modify LDL, leading to the generation of lipid peroxidation products and reactive oxygen species (ROS). Peroxidation of