UNIVERSITI TEKNOLOGI MARA

EFFECTS AND MECHANISM OF ACTION OF TOCOTRIENOL RICH FRACTION (TRF) IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) USING DIETARY INDUCED AND GENETICALLY MODIFIED ANIMAL MODEL

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PhD

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AUTHOR'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 results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver disease encompassing benign liver steatosis to severe conditions such as steatohepatitis and cirrhosis. It occurs due to constant oxidative stress and inflammation. NAFLD prevalence has increased up to 27% from 2011 to 2015 globally. To date, there is no FDA-approved drug for its treatment, although vitamin E given to patients exhibited varying degrees of success. Tocotrienol has been shown to be a more potent antioxidant among the isomers of vitamin E but is less absorbed. Therefore, in this study, the effect of a new formulation of palm-based tocotrienol rich fraction (ETRF) in reducing NAFLD development using animal models was compared to the original TRF formulation. ETRF has a higher content of tocotrienol in medium-chain triglyceride (MCT) carrier while TRF was in long-chain triglyceride (LCT) carrier. Two NAFLD models were used: NAFLD induced by high fat diet (HFD) intake in ICR male mice (ICR NAFLD model) and leptin-knockout B6.Cg-LepOb/J male mice NAFLD (JAX NAFLD model). Mice were fed with HFD mixed with TRF or ETRF (200 mg/kg/day). The ICR mice were divided into standard diet (SD), HFD, HFD with TRF and HFD with ETRF. Both SD and HFD are control groups. The JAX mice were divided into HFD, HFD with palm-kernel oil (PKO), HFD with TRF and HFD with ETRF. The supplementation period was carried out for ten weeks for ICR NAFLD model and six weeks for JAX NAFLD model. Anthropometric indices, random blood glucose (RBG), liver histology, liver superoxide dismutase (SOD) activity, liver total glutathione (GSH) activity and liver Farnesoid-X receptor (FXR) expression were assessed. Serum was subjected to untargeted metabolomic analysis using UHPLC-Orbitrap. Significantly lower total body weight changes, higher food intake and food intake to body weight ratio were found in TRF supplemented models compared to other groups ($p<0.05$). No significant changes were observed on abdominal circumference and random blood glucose measured in all groups ($p>0.05$). Smaller liver span ($p<0.05$) and lower NAFLD activity score (NAS) were recorded in JAX mice supplemented with ETRF. In metabolomics, principal component analysis showed better distinction between groups in JAX NAFLD model compared to ICR NAFLD model. Metabolites profiled in ETRF JAX demonstrated downregulation of primary and secondary bile acids supported by upregulation of FXR protein expression ($p<0.05$). ETRF JAX showed upregulation of N6,N6, and N6-Trimethyl-L-lysine and downregulation of L-acetylcarnitine ($p<0.05$), indicating increased β-oxidation. Metabolites in purine salvage and degradation pathways were also upregulated in ETRF JAX ($p<0.05$). Downregulation of pro-inflammatory metabolites including sphingolipid, allantoic acid and arachidonic acid ($p<0.05$) were observed in TRF and ETRF JAX. In contrast, upregulation of anti-inflammatory metabolite trigonelline was observed in TRF and ETRF ($p<0.05$). In conclusion, ETRF improves liver histology and limits liver enlargement which could be mediated by its ability to regulate FXR, bile acids metabolism, anti-inflammatory pathways, ketogenesis, purine salvage and degradation pathways and β-oxidation. Although TRF showed similar outcomes, the effects were not profound compared to ETRF as TRF acted upon peripheral tissues while ETRF showed positive effects on liver. These findings contribute to the body of knowledge on TRF/ETRF, in term of its formulation, regulations of metabolites, and as a promising agent to improve NAFLD. Metabolomics findings from this study provides an insight of TRF/ETRF in deregulating the important metabolites in NAFLD.

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