Journal of Functional Foods 26 (2016) 338–349

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

Nanoemulsified gamma-oryzanol rich fraction blend regulates hepatic cholesterol metabolism and cardiovascular disease risk in hypercholesterolaemic rats

Aminu Ishaka [a,b], *Mustapha Umar Imam* [c], *Maznah Ismail* [a,*], *Rozi Mahmud* [d], *Zuki Zakaria Abu Bakar* [e]

[a] *Laboratory of Molecular Biomedicine, Institute of Bioscience, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

[b] *Department of Medical Biochemistry, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria*

[c] *Precision Nutrition Innovation Institute, College of Public Health, Zhengzhou University, Zhengzhou 450001 Henan Province, China*

[d] *Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Malaysia*

[e] *Faculty of Veterinary Medicine, University Putra Malaysia, 43400 Serdang, Malaysia*

ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 25 July 2016
Accepted 8 August 2016
Available online

Keywords:
Cardioprotection
Gamma-oryzanol rich fractions–palm oil blend nanoemulsion
Lipid parameter
F_2-isoprostane
Heart weight
Hepatic cholesterol metabolism

ABSTRACT

We have reported that a bioactive-rich fraction, called EORY, from a combination of supercritical fluid-extracted rice bran oil and palm oil has abundant gamma-oryzanol, tocopherols and tocotrienol. Moreover, EORY has balanced composition of polyunsaturated:saturated fatty acids, with the potential for hypocholesterolaemic and antioxidant effects. However, the bioactive compounds in EORY are lipophilic and therefore pose bioavailability problems. This study evaluated the cardioprotective effects of orally-administered EORY emulsion and its nanoemulsion (NEORY) on diet-induced hypercholesterolaemic rats. NEORY reduced body weight gain, heart weight, lipid parameters and oxidised LDL, and improved HDL better than EORY and simvastatin. NEORY also significantly increased hepatic mRNA expression of HMGCoA reductase, apolipoprotein A1 and LDLR, and lowered apolipoprotein B and LPL. The effects of NEORY on lipid parameters, lipid peroxidation markers and hepatic cholesterol metabolism suggested that it could regulate the risk of cardiovascular disease, possibly due to increased absorption of gamma-oryzanol, tocopherols and tocotrienol.

* *Corresponding author.* Laboratory of Molecular Biomedicine, Institute of Bioscience, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia. Fax: +603 8947 2116.

E-mail addresses: maznahis@upm.edu.my, myhome.e@gmail.com (M. Ismail).

Abbreviations: ABCA, ATP-binding cassette; BF3, boron trifluoride; CVD, cardiovascular disease; EORY, gamma-oryzanol-rich fraction and palm oil blend; HCD, high-cholesterol diet; HDL, high-density lipoprotein; HMGCoA, 3-hydroxy-3-methyl-glutaryl-CoA; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPL, lipoprotein lipase; MUFA, monounsaturated fatty acids; ND, normal diet; NEORY, EORY nanoemulsion; ORF, oryzanol rich fraction; ox-LDL, oxidised LDL; PO, palm oil; PPARγ, peroxisome proliferator-activated receptor gamma; PUFA, polyunsaturated fatty acids; RBO, rice bran oil; SFA, saturated fatty acids; SFE, supercritical extraction; SMV, simvastatin; TC, total cholesterol; TG, triacylglycerols

http://dx.doi.org/10.1016/j.jff.2016.08.015
1756-4646/

1. Introduction

There has been an emerging interest in compounds that occur naturally in plants and their extracts as alternative therapies and risk-minimising agents for chronic diseases such as cardiovascular disease (CVD) disease (Frank, Andrews, Elliott, Lew, & Boston, 2005; Stevinson, Pittler, & Ernst, 2000; Temple, 2000). In recent years, the management of CVD has attracted considerable attention, including the exploration of newer therapies to minimise its risk and the associated mortality. Many cases of CVD have dyslipidaemia as a hallmark, owing to changes in lipid metabolism and control (Leon & Bronas, 2009). In this regard, CVD management targets lowering low-density lipoprotein (LDL) and triacylglycerols (TG), and increasing the level of high-density lipoprotein (HDL), which clears cholesterol from tissues by reverse transport. In addition, many therapies target complex molecular cholesterol metabolism regulatory mechanisms, including the modulation of the genes for 3-hydroxy-3-methyl-glutaryl-CoA (HMGC) reductase, adenosine triphosphate-binding cassette transporter (ABCA1), peroxisome proliferator-activated receptor gamma (PPARγ), apolipoprotein A1, apolipoprotein B, lipoprotein lipase (LPL), low-density lipoprotein receptor (LDLR) and leptin (Lee, Olson, & Evans, 2003; Mäkynen, Chitchumroonchokchai, Adisakwattana, Failla, & Ariyapitipun, 2012; Oram & Vaughan, 2006; Otarod & Goldberg, 2004). Oxidative stress plays an important role in the development of CVD through an increase in oxidised low-density lipoprotein (ox-LDL) and the resulting inflammation, which makes the ox-LDL level an indicator of the risk of CVD development (Holvoet, De Keyzer, & Jacobs, 2008; Shen et al., 2013).

Rice bran, a product of rice milling, has attracted the interest of researchers due to its nutritional and biological effects. It contains rice bran oil (RBO), which is extracted by supercritical fluid extraction (SFE) among other methods of extraction (Vagi, Simándi, Daood, Deak, & Sawinsky, 2002). SFE using carbon dioxide as an alternative method to conventional ohmic heating and organic solvent extraction produces, under the optimised conditions, an oryzanol-rich fraction (ORF) rice bran oil (RBO) (Ismail, Al-Naqeeb, Mamat, & Ahmad, 2010; Johnson & Lusas, 1983; Lakkakula, Lima, & Walker, 2004). The ORF is not only rich in gamma-oryzanol (a mixture of phytosteryl ferulates and triterpene alcohols), but also consists of significant amounts of tocopherols, tocotrienols, phytic acid, lecithin and inositol, which are potent antioxidants. Gamma-oryzanol has, among other qualities, antioxidant, cholesterol-lowering and antiplatelet aggregation effects (Cicero & Gaddi, 2001; Hiramitsu & Armstrong, 1991; Ismail et al., 2010).

Furthermore, palm oil (PO), which is expressed from fresh sun basket fruit, has been reported to contain significant amounts of saturated fatty acids, tocopherols, tocotrienols and carotenoids (Kruger, Engelbrecht, Esterhuyse, du Toit, & van Rooyen, 2007). In the past we have demonstrated that a bioactive-rich fraction, which was called EORY, a mixture of SFE-extracted ORF and PO had balanced saturated-to-unsaturated fatty acid ratio of 0.67 and a high amount of oryzanol and total tocols (Ismail, 2011). The composition of EORY is highly suggestive of excellent nutritional qualities and biological value (Chopra, Kumari, & Nagraj, 2004; Reena & Lokesh, 2007). However, there are issues with the absorption and bioavailability of some of the bioactive components such as gamma-oryzanol and tocols (Heinemann, Axtmann, & Bergmann, 1993; Idris et al., 2014; Ismail & Loh, 2005; Packer, Weber, & Rimbach, 2001; Yap, Yuen, & Lim, 2003), due to the fact that water-insoluble substances, which form most of the bioactive components of EORY, characteristically have low dissolution and hence poor bioavailability (Chu, Ichikawa, Kanafusa, & Nakajima, 2008). To circumvent this limitation, nanoemulsion formulations can be used to improve and control the delivery of water-insoluble pharmaceuticals, nutraceuticals and cosmeceuticals (Mason, Wilking, Meleson, Chang, & Graves, 2006).

In this study, the effects of orally administered EORY and its nanoemulsion (NEORY) on diet-induced hypercholesterolaemia in rats were evaluated over an 8-week treatment period, in comparison with simvastatin treatment and controls. The effects on the lipid profile, serum ox-LDL, F_2-isoprostane, heart weight and hepatic cholesterol metabolism genes were compared.

2. Materials and methods

2.1. Materials

Rice bran was obtained from the Bernas milling factory at Kuala Selangor, Selangor, Malaysia. Commercially available palm oil (Seri Murni, PGEO Edible Oils Sdn. Bhd. Pasir Gudang, Johor, Malaysia) was purchased for the blend (EORY). All the reagents and solvents used were either analytical grade or HPLC grade and were purchased from Merck (Darmstadt, Germany). The standards for gamma-oryzanol, tocopherols and tocotrienols were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lipid profile kits (LDL, HDL, total cholesterol [TC], and TG) were purchased from Randox Laboratories Ltd. (Crumlin, County Antrim, UK). Ox-LDL and F_2-isoprostane kits were purchased from Cusabio Biotech Co., Ltd. (Wuhan, China) and Cayman Chemicals (Ann Arbor, MI, USA), respectively, while the GenomeLab™ GeXP Start Kit was purchased from Beckman Coulter Inc. (Miami, FL, USA).

2.2. ORF extraction

About 160 g of freshly obtained rice bran, stabilised by heating using an automated microwave oven (2450 MHz, 550 Watts, 110 °C for 200 sec) and stored at 4 °C was extracted using an SFE machine (Thar 1000F, Thar Technologies, Inc., Pittsburgh, PA, USA) for 3 h at 60 °C temperature, 600 bars pressure and carbon dioxide flow rate of 25 g/min. In addition, fractionation was done at 30 °C and 300 bars. The extracted oil was collected and the percentage yield was calculated with respect to the rice bran sample weight.

2.3. EORY formation

EORY was formed by mixing SFE-extracted ORF and PO in a ratio of 60:40 (v/v). The mixture was continuously stirred to blend completely and then stored at 4 °C until further analysis.

2.3.1. EORY bioactive analyses

The bioactive analyses for EORY were done by determining its gamma-oryzanol, total tocol (tocopherols and tocotrienols) and