



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF ORYZANOL AND TOCOTRIENOL ON PLATELET
AGGREGATION AND BLOOD LIPID PROFILE IN RATS**

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By

NURZILLAH BINTI MALIKI

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EFFECTS OF ORYZANOL AND TOCOTRIENOL ON PLATELET AGGREGATION AND BLOOD LIPID PROFILE IN RATS

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August 2007

Chairman : Professor Maznah Ismail, PhD

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Rice bran is the outer brown layer of the rice kernel that is removed during the milling process to produce white rice. The bran portion of the rice kernel is one of the most nutritious portions of the kernel. Recently, there are evidences suggesting that key components of rice may play a role in health maintenance and disease prevention.

Fully-processed rice bran oil contains higher amount of unsaponifiable components than most vegetable oils. The focus has been on oryzanol and vitamin E, especially tocotrienols which were found to have many health benefits. Thus, this project was undertaken to determine the effect of oryzanol in combination with tocotrienol on platelet aggregation, plasma lipid profile, kidney and liver function parameters and the histology of the aorta in rats.



A total of 140 male Sprague-Dawley rats with body weights ranging from 230 to 280 g were divided into 2 treatment batches (n=70/batch). The first batch received intervention treatment while receiving a high cholesterol diet. The second batch was given a high cholesterol diet for one month before treatment and followed by normal rat chow at the time the dietary treatment was instituted.

In the first treatment batch, a total of 70 rats were randomly distributed into 7 groups (n=10/group); Control, HCD (Control + 1% cholesterol + 0.15% cholic acid), HCD + ASA (HCD + 0.5% aspirin), HCD + ORY (HCD + 0.5% oryzanol in triolein), HCD + TRF (HCD + 0.5% tocotrienol-rich fraction in triolein), HCD + OT (HCD + 0.5% oryzanol + tocotrienol in triolein) and HCD + EMUL (HCD + 0.5% oryzanol + tocotrienol emulsion). Each group of animals was fed one type of diet treatment only and allowed free access to water throughout the study period. Treatments were applied by oral gavage for 8 weeks. Blood samples were collected twice throughout this study; at 0 week, 4 weeks and 8 weeks of treatment.

The second treatment batch on the other hand received intervention diets after hypercholesterolemia induction. They were also randomly distributed into 7 groups (n=10/group); Control, HCD (Control + 1% cholesterol + 0.15% cholic acid), ASA (0.5% aspirin), ORY (0.5% oryzanol in triolein), TRF (0.5% tocotrienol-rich fraction in triolein), OT (0.5% oryzanol + tocotrienol in triolein) and EMUL (0.5% oryzanol + tocotrienol emulsion). All groups were fed with high cholesterol diet (normal + 1% cholesterol + 0.15% cholic acid) for 4 weeks except for Control group, which was fed with normal rat chow. The hypercholesterolemic rats were then orally treated for 8

weeks. The blood samples were collected 4 times throughout this experiment; at the beginning of the experiment (pre-induction week), 4 weeks after induction with cholesterol (0 week) and at 4 and 8 weeks of treatment.

At the termination of the experiment, the rats were weighed and blood was collected by cardiac puncture. Complete autopsies were performed after the rats had been sacrificed. The rats were dissected and the aortas removed, opened longitudinally, and prepared for detection and estimation of lipid deposits in the intima. The part of the aorta proximal to the heart was cut, labeled, fixed in 10% formalin and prepared for light microscopy examination hematoxylin and eosin (H & E).

Whole blood was analysed for platelet aggregation. The total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), and triglyceride (TG), alanine aminotransferase (ALT), γ -glutamyltransferase (GGT), urea, and creatinine plasma concentrations were also analysed.

The present study demonstrates that all treatments (ASA, ORY, TRF, OT and EMUL) reduced plasma TC and LDL concentrations and inhibit platelet aggregation in rats. The oryzanol and tocotrienol combination showed the highest inhibition on platelet aggregation in the first treatment batch by -42.33%, -35.94%, and -61.40% and in the second batch by -54.04%, -57.80%, and -69.20% with 10 μ l adenosine-5'-diphosphate (ADP), 20 μ l ADP, and 20 μ l collagen respectively. The results from this study have shown that the combination of oryzanol and tocotrienol is potentially a good hypocholesterolemic agent. In addition, treatment with combination of oryzanol and

tocotrienol in triolein showed significant decreases ($p < 0.05$) in plasma TC and LDL concentrations in first batch of rats by -10% and -36% and in the second batch by -37.5% and -73.49% respectively.

Treatment with oryzanol either oryzanol plus tocotrienol in triolein or oryzanol plus tocotrienol emulsion decreased the concentrations of kidney (urea and creatinine) and liver (ALT and GGT) function parameters suggesting that there is no toxic effect on the kidneys or liver. Histological assessment also showed that the blood vessel tissues were not affected by the treatment. No lipid deposit was detected in the aorta of rats.

In summary, these studies suggested that in hypercholesterolemic rats the combination of oryzanol and tocotrienol have a synergistic effect. The results indicated that various components of rice bran have potential as anti-platelet aggregation and hypocholesterolemic agents. Therefore, the synergistic properties of oryzanol and tocotrienol could play an important role in reducing the risk of development of cardiovascular disease.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN ORIZANOL DAN TOKOTRIENOL KE ATAS PENGAGREGATAN
PLATELET DAN PROFIL LIPID DARAH TIKUS**

Oleh

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Dedak beras merupakan lapisan luar padi berwarna perang yang terhasil semasa proses pengisaran beras putih. Dedak beras tersebut merupakan salah satu bahagian isirung padi yang paling bernutrien. Walau bagaimanapun, kini ada bukti yang menyarankan komponen utama beras boleh memainkan peranan dalam penjagaan kesihatan dan pencegahan penyakit.

Minyak dedak beras terproses mengandungi komponen tak boleh sabun lebih tinggi daripada minyak sayuran. Tumpuan adalah terhadap orizanol dan vitamin E, terutamanya tokotrienol yang didapati mempunyai banyak faedah kesihatan. Oleh itu, projek ini dijalankan untuk menentukan kesan gabungan orizanol dan tokotrienol ke atas pengagregatan platelet, profil lipid plasma, parameter fungsi ginjal dan hati serta histologi aorta tikus.



Sejumlah 140 ekor tikus Sprague-Dawley berat antara 230 hingga 280 g telah dibahagikan kepada dua kelompok rawatan (n=70/kelompok). Kelompok pertama menerima rawatan sambil menerima diet kolesterol tinggi. Kelompok kedua diberi diet kolesterol tinggi selama satu bulan sebelum rawatan dan diberi makanan tikus normal ketika rawatan diet dimulakan.

Dalam kelompok rawatan pertama, 70 ekor tikus diletak secara rambang ke dalam 7 kumpulan (n=10/kumpulan); Kawalan, HCD (Kawalan + 1% kolesterol + 0.15% asid kolik), HCD + ASA (HCD + 0.5% aspirin), HCD + ORY (HCD + 0.5% orizanol dalam triolein), HCD + TRF (HCD + 0.5% tokotrienol dalam triolein), HCD + OT (HCD + 0.5% orizanol + tokotrienol dalam triolein) dan HCD + EMUL (HCD + 0.5% emulsi orizanol + tokotrienol). Setiap kumpulan haiwan tersebut menerima satu jenis diet rawatan sahaja dan minuman yang tidak dihadkan sepanjang kajian dijalankan. Rawatan diberikan secara gavaj oral selama 8 minggu. Darah diperolehi 3 kali sepanjang kajian; pada 0 minggu, 4 minggu, dan 8 minggu rawatan.

Kelompok rawatan kedua sebaliknya menerima diet rawatan selepas aruhan hiperkolesterolemia. Kelompok ini juga diletakkan secara rambang ke dalam 7 kumpulan (n=10/kumpulan); Kawalan, HCD (Kawalan + 1% kolesterol + 0.15% asid kolik), ASA (0.5% aspirin), ORY (0.5% orizanol dalam triolein), TRF (0.5% tokotrienol dalam triolein), OT (0.5% orizanol + tokotrienol dalam triolein) and EMUL (0.5% emulsi orizanol + tokotrienol). Semua kumpulan menerima diet tinggi kolesterol (normal + 1% kolestrol + 0.15% asid kolik) selama 4 minggu kecuali kumpulan Kawalan yang diberi makanan tikus normal. Tikus hiperkolesterolemia kemudian diperlakukan secara

rawatan oral selama 8 minggu. Sampel darah diambil sebanyak 4 kali sepanjang ujikaji; pada permulaan ujikaji (minggu pra-pengaruh), 4 minggu selepas pengaruh dengan kolesterol (minggu 0) dan pada 4 dan 8 minggu rawatan.

Di akhir ujikaji, tikus ditimbang dan darah diperolehi melalui tebuk kardium. Autopsi lengkap dilakukan selepas tikus dimatikan. Tikus tersebut didisek dan aortanya dikeluarkan, dibuka secara longitud, dan disediakan untuk pengesanan dan penentuan enapan lipid dalam intima. Bahagian aorta yang paling hampir dengan jantung dipotong, dilabel, ditetapkan dalam 10% formalin, disediakan untuk pemeriksaan mikroskopi cerah hematoxylin and eosin (H&E).

Darah sepenuh dianalisis untuk pengagregatan platelet. Kepekatan kolesterol sepenuh (TC), lipoprotein ketumpatan rendah (LDL), lipoprotein ketumpatan tinggi (HDL), dan trigliserida (TG), alanina aminotransferase (ALT), γ -glutamyltransferase (GGT), urea, dan kreatinin plasma juga dianalisis.

Kajian ini menunjukkan semua jenis rawatan (ASA, ORY, TRF, OT and EMUL) telah mengurangkan kepekatan TC dan LDL plasma serta merencat pengagregatan platelet dalam tikus. Gabungan orizanol dan tokotrienol menunjukkan penurunan pengagregatan platelet yang paling tinggi pada kelompok rawatan pertama masing-masing sekadar -42.33%, -35.94% dan -61.40% dan pada kelompok rawatan kedua masing-masing sekadar -54.04%, -57.80% dan -69.20% dengan 10 μ l adenosine-5'-diphosphate (ADP), 20 μ l ADP, dan 20 μ l kolagen. Hasil kajian ini menunjukkan gabungan orizanol dan tokotrienol berpotensi untuk menjadi agen hipokolesteromia yang baik. Selain itu,

rawatan dengan gabungan orizanol dan tokotrienol dalam triolein menunjukkan penurunan tererti ($p < 0.05$) dalam kepekatan TC dan LDL plasma pada tikus kelompok rawatan pertama masing-masing sekadar -10% , -36% and pada tikus kelompok rawatan kedua masing-masing sekadar -37.5% dan -73.49% .

Rawatan dengan orizanol sama ada orizanol dan tokotrienol dalam triolein atau emulsi orizanol dan tokotrienol menurunkan kepekatan parameter fungsi ginjal (urea dan kreatinin) dan hati (ALT dan GGT), menyarankan tiada kesan toksik terhadap ginjal atau hati. Penilaian histologi juga menunjukkan tisu aorta tidak terjejas kerana rawatan tersebut. Enapan lipid tidak terkesan dalam aorta tikus.

Kesimpulannya, kajian ini menyarankan dalam tikus hiperkolesterolemia, gabungan orizanol dan tokotrienol memberi kesan sinergi. Hasil kajian menunjukkan pelbagai komponen dedak beras berpotensi sebagai agen hipokolesterolemia dan anti-pengagregatan platelet. Dengan ini, sifat sinergi orizanol dan tokotrienol boleh memainkan peranan penting dalam mengurang risiko berlakunya penyakit kardiovaskular.

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I certify that an Examination Committee met on 29th August 2007 to conduct the final examination of Nurzillah Binti Maliki on her Master of Science thesis entitled “The Effect of Oryzanol and Tocotrienol on Platelet Aggregation and Blood Lipid Profile in Rats” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree.

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DECLARATION

I declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NURZILLAH MALIKI

Date : 3 January 2008



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LIST OF ABBREVIATIONS

Acetyl-CoA	Acetyl coenzyme A
ACUC	Animal care and use committee
ADP	Adenosine-5'-Diphosphate
ALA	Alpha-lipoic acid
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AP	Alkaline phosphatase
APO A	Apolipoprotein A
(APO) B	Apolipoprotein B
ASA	Aspirin
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CAD	Coronary artery disease
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
EMUL	Emulsion
EPA	Ecosapentaenoic acid
FFA	Free fatty acid



GGT	Gamma-glutamyltranspeptidase
GNO	Groundnut oil
GSH	g-glutamyl cysteinylglycine
H & E	Hematoxylin and eosin
HCD	High cholesterol diet
HCl	Hydrochloric acid
HDL-C	High-density lipoprotein cholesterol
HMG-CoA	Hydroxyl methylglutamyl coenzyme A
IDL	Intermediate-density lipoprotein
LD	Lactate dehydrogenase
LDL-C	Low-density lipoprotein cholesterol
LPL	Lipoprotein lipase
NCEP	National Cholesterol Education Program
ORY	Oryzanol
OT	Oryzanol + tocotrienol-rich fraction
PPP	Platelet poor plasma
PRP	Platelet rich plasma
RBO	Rice bran oil
SD	Standard deviation
SPSS	Statistical package for social science
TC	Total cholesterol
TG	Triglycerides
TRF	Tocotrienol-rich fraction