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

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By

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August 2007

Chairman : Professor Maznah Ismail, PhD
Faculty : Medicine and Health Sciences

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 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), \( \gamma \)-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 \( \mu \)l adenosine-5’-diphosphate (ADP), 20 \( \mu \)l ADP, and 20 \( \mu \)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

NURZILLA MALIKI

Ogos 2007

Pengerusi : Profesor Maznah Ismail, PhD

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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 memain 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 + tocotrienol). 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 + tocotrienol). Semua kumpulan menerima diet tinggi kolesterol (normal + 1% kolesterol + 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 pengesan 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-glutamiltransferase (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 kelo mpok rawatan kedua masing-masing sekadar  -54.04%, -57.80% dan –69.20% dengan 10 µl adenosine-5’-diphosphate (ADP), 20 µl ADP, dan 20 µl kolagen. Hasil kajian ini menunjukkan gabungan orizanol dan tokotrienol berpotensi untuk menjadi agen hipokolesterolomia 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.

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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.

Members of the Examination Committee were as follows:

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Date:
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date : 21 February 2008
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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4.21 The intimal surface of the aorta from the ASA group

4.22 The intimal surface of the aorta from the ORY group

4.23 The intimal surface of the aorta from the TRF group

4.24 The intimal surface of the aorta from the OT group

4.25 The intimal surface of the aorta from the EMUL group

4.26 Hematoxylin and eosin stained (H&E) of the intimal thickening of aorta as shown by image-analysis system interfaced to a Zeiss Axioscop microscope (x20) in Control group
4.27 Hematoxylin and eosin stained (H&E) of the intimal thickening of aorta as shown by image-analysis system interfaced to a Zeiss Axioscop microscope (x20) in HCD group

4.28 Hematoxylin and eosin stained (H&E) of the intimal thickening of aorta as shown by image-analysis system interfaced to a Zeiss Axioscop microscope (x20) in ASA group

4.29 Hematoxylin and eosin stained (H&E) of the intimal thickening of aorta as shown by image-analysis system interfaced to a Zeiss Axioscop microscope (x20) in ORY group

4.30 Hematoxylin and eosin stained (H&E) of the intimal thickening of aorta as shown by image-analysis system interfaced to a Zeiss Axioscop microscope (x20) in TRF group

4.31 Hematoxylin and eosin stained (H&E) of the intimal thickening of aorta as shown by image-analysis system interfaced to a Zeiss Axioscop microscope (x20) in OT group

4.32 Hematoxylin and eosin stained (H&E) of the intimal thickening of aorta as shown by image-analysis system interfaced to a Zeiss Axioscop microscope (x20) in EMUL group
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>Acetyl-CoA</td>
<td>Acetyl coenzyme A</td>
</tr>
<tr>
<td>ACUC</td>
<td>Animal care and use committee</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine-5’-Diphosphate</td>
</tr>
<tr>
<td>ALA</td>
<td>Alpha-lipoic acid</td>
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<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>AP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>APO A</td>
<td>Apolipoprotein A</td>
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<tr>
<td>(APO) B</td>
<td>Apolipoprotein B</td>
</tr>
<tr>
<td>ASA</td>
<td>Aspirin</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated hydroxyanisole</td>
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<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
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<tr>
<td>CETP</td>
<td>Cholesteryl ester transfer protein</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>EDTA</td>
<td>Ethylene diamine tetra acetic acid</td>
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<td>EMUL</td>
<td>Emulsion</td>
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<td>EPA</td>
<td>Ecosapentaenoic acid</td>
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<td>FFA</td>
<td>Free fatty acid</td>
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<td>Definition</td>
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<tr>
<td>GGT</td>
<td>Gamma-glutamyltranspeptidase</td>
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<td>GNO</td>
<td>Groundnut oil</td>
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<td>GSH</td>
<td>g-glutamyl cysteinylglycine</td>
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<td>H &amp; E</td>
<td>Hematoxylin and eosin</td>
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<td>HCD</td>
<td>High cholesterol diet</td>
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<td>HCI</td>
<td>Hydrocholic acid</td>
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<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
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<td>HMG-CoA</td>
<td>Hydroxyl methylglutamyl coenzyme A</td>
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<tr>
<td>IDL</td>
<td>Intermediate-density lipoprotein</td>
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<td>LD</td>
<td>Lactate dehydrogenase</td>
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<td>LPL</td>
<td>Lipoprotein lipase</td>
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<td>NCEP</td>
<td>National Cholesterol Education Program</td>
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<tr>
<td>ORY</td>
<td>Oryzanol</td>
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<td>OT</td>
<td>Oryzanol + tocotrienol-rich fraction</td>
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<td>PPP</td>
<td>Platelet poor plasma</td>
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<tr>
<td>PRP</td>
<td>Platelet rich plasma</td>
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<td>RBO</td>
<td>Rice bran oil</td>
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<td>Statistical package for social science</td>
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<td>Total cholesterol</td>
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<tr>
<td>TG</td>
<td>Triglycerides</td>
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<td>TRF</td>
<td>Tocotrienol-rich fraction</td>
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