UNIVERSITI PUTRA MALAYSIA

EFFECTS OF PHYTIC ACID EXTRACTED FROM RICE BRAN ON AZOXYMETHANE-INDUCED COLON CARCINOGENESIS IN RATS

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By

NORAZALINA SAAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Sciences

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Specially dedicated to

My mum and sister

For their invaluable love, understanding, encouragement and patience.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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April 2008

Chairman : Norhaizan Mohd Esa, PhD

Faculty : Medicine and Health Sciences

This research is carried out to study the potential of phytic acid extracted from rice bran in the suppression of colon carcinogenesis in rats. In the optimization of phytic acid extraction, results showed 5% H_2SO_4 in pH 0.6 and 30 minutes of extraction time gave the highest amount of phytic acid. In animal study, 72 male *Sprague-Dawley* rats were divided into 6 groups with 12 rats in each group; Group 1: AOM alone, Group 2: AOM + 0.2% (w/v) Commercial Phytic Acid (CPA), Group 3: AOM + 0.5% (w/v) Commercial Phytic Acid (CPA), Group 4: AOM + 0.2% (w/v) Extract Phytic Acid (EPA), Group 5: AOM + 0.5% (w/v) Extract Phytic Acid (EPA). Rats received two subcutaneous injections of azoxymethane (AOM) in saline at (15mg/kg bodyweight) over a 2-weeks period to induce colon cancer. The treatments were given in two different concentrations of phytic acid; 0.2% (w/v) and 0.5% (w/v) during post initiation of carcinogenesis phase via drinking water.

The colons of the animals were analyzed for detection and quantification of aberrant crypt foci (ACF) after 8 weeks of treatment. The finding showed treatment with 0.2% (w/v) EPA gave the greatest reduction in the formation of ACF. In addition, phytic acid significantly suppressed the number of ACF in the distal, middle and proximal colon as compared to AOM alone ($p<0.05$). For the histological classification of ACF, treatment with 0.5% (w/v) CPA had the highest percentage (71%) of non-dysplastic ACF followed by treatment with 0.2% (w/v) EPA (61%). After 20 weeks of treatment, colons of the rats were excised and analyzed for tumor incidence. Results showed that administration of phytic acid reduced the incidence and multiplicity of total tumors and adenocarcinomas even though there were no significant differences between groups.

For the immunohistochemical analyses, proliferating cell using Ki-67 and modulating of ß-catenin and COX-2 expression were assessed as those have been shown to play a role in tumor progression. In Ki-67, there was a statistically significance difference in lowering the proliferating index between treatment groups as compared to AOM alone ($p<0.05$). For ß-catenin and COX-2 expression, there was a significant difference between groups as ($p=0.000$) and ($p=0.030$). In the correlation test, the results showed that there was a significant positive correlation ($p=0.010$) between proliferation of Ki67 and COX-2 expression. A positive linear relationship was found between total Ki67 and ß-catenin but these relationships were not statistically significant. Total ß-catenin had a significant positive linear relationship with total COX-2 ($p = 0.044$).

As a conclusion, this study found the potential value of phytic acid extracted from rice bran in reducing colon cancer risk in rats. Besides identification of cancer reduction strategies based on dietary modification including looking at natural sources that may have anticancer properties, an alternative compound from local sources has been developed. Therefore, rice bran that is normally discarded as by-product of rice production will increase in value due to phytic acid potential as a nutraceutical compound in the prevention of colon cancer progression.

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

KESAN ASID FITIK YANG DIEKSTRAK DARIPADA DEDAK BERAS TERHADAP TIKUS YANG TERARUH KANSER KOLON MENGGUNAKAN AZOKSIMETANA

Oleh

Norazalina Saad

April 2008

Pengerusi : Norhaizan Mohd Esa, PhD

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Kajian ini dijalankan dengan tujuan untuk mengkaji potensi asid fitik yang diekstrak daripada dedak beras dalam mengurangkan kejadian kanser kolon pada tikus. Dalam mendapatkan tahap pengekstrakan asid fitik, keputusan menunjukkan 5% H_2SO_4 dengan pH 0.6 dan masa pengekstrakan selama 30 minit telah memberikan jumlah amaun asid fitik yang tinggi. Dalam kajian haiwan eksperimen, 72 ekor tikus spesis *Sprague-Dawley* dibahagikan kepada 6 kumpulan dengan setiap kumpulan mempunyai 12 ekor; Kumpulan 1: Azoksimetana (AOM) sahaja, Kumpulan 2: AOM + 0.2% (w/v) Komersial Asid Fitik (CPA), Kumpulan 3: AOM + 0.5% (w/v) Komersial Asid Fitik (CPA), Kumpulan 4: AOM + 0.2% (w/v) Ekstrak Asid Fitik (EPA), Kumpulan 5: AOM + 0.5% (w/v) Ekstrak Asid Fitik (EPA), Kumpulan 6: Normal. Tikus telah menerima

dua suntikan AOM secara intraperitoneum dalam larutan garam yang steril pada kepekatan (15mg/kg berat badan) selama 2 minggu untuk mengaruh kolon kanser. Rawatan asid fitik diberi dalam dua kepekatan yang berbeza; 0.2% (w/v) dan 0.5% (w/v) selepas fasa permulaan karsinogenesis melalui air minuman.

Kolon daripada haiwan eksperimen dianalisa untuk mengesan dan mengira focus kript aberan (ACF) selepas rawatan selama 8 minggu. Keputusan menunjukkan bahawa rawatan dengan 0.2% (w/v) EPA memberikan kadar penurunan yang paling tinggi dalam pembentukan ACF. Selain itu, asid fitik secara signifikan merendahkan jumlah ACF pada kolon di bahagian distal, pertengahan dan proksimal dibandingkan dengan kumpulan AOM sahaja. Untuk pengelasan histologikal ACF, rawatan dengan 0.5% (w/v) CPA mempunyai peratus tertinggi (71%) ACF tisu yang tidak mengalami displasia diikuti oleh rawatan dengan menggunakan 0.2% (w/v) EPA (61%). Selepas rawatan selama 20 minggu, kolon tikus dikeluarkan dan dianalisa untuk melihat pembentukan tumor. Keputusan menunjukkan bahawa pemberian asid fitik boleh mengurangkan kejadian dan penggandaan jumlah tumor dan adenokarsinoma walaupun tidak terdapat perbezaan yang signifikan di antara kumpulan.

Untuk analisis menggunakan kaedah immunohistokimia, penentuan proliferasi sel Ki-67 dan modulasi ekspresi ß-catenin dan COX-2 digunakan untuk menunjukkan peranan dalam perkembangan tumor. Dalam Ki-67, terdapat perbezaan yang signifikan dalam merendahkan indeks proliferasi antara

kumpulan rawatan apabila dibandingkan dengan AOM sahaja ($p<0.05$). Dalam ekspresi ß-catenin dan COX-2, terdapat perbezaan yang signifikan antara kumpulan ($p=0.000$) dan ($p=0.030$). Dalam ujian korelasi, keputusan menunjukkan terdapat korelasi positif yang signifikan ($p=0.010$) antara perkembangan proliferasi Ki67 dan ekspresi COX-2. Didapati hubungan yang linear positif antara keseluruhan ekspresi Ki67 dan ß-catenin, namun perkaitan adalah tidak signifikan. Keseluruhan ß-catenin mempunyai hubungan linear positif yang signifikan dengan jumlah expresi COX-2 ($p=0.044$).

Sebagai kesimpulan, kajian ini menunjukkan nilai potensi asid fitik yang diekstrak daripada dedak beras dalam mengurangkan risiko kanser kolon pada tikus. Selain penentuan strategi untuk mengurangkan kanser berdasarkan modifikasi diet dengan memberi perhatian kepada sumber semula jadi yang mempunyai sifat anti kanser, sebatian alternatif daripada sumber tempatan dapat dihasilkan. Dengan itu, dedak beras yang biasanya dibuang sebagai hasil sampingan dalam penghasilan beras akan dapat ditingkatkan nilainya dengan adanya asid fitik yang berpotensi sebagai agen nutraseutikal dalam pencegahan perkembangan kolon kanser.

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I certify that an Examination Committee has met on 27 August 2008 to conduct the final examination of Norazalina Saad on her Master of Science thesis entitled "Effects of Phytic Acid Extracted from Rice Bran on Azoxymethane-Induced Colon Carcinogenesis 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 student be awarded the Master of Science.

Members of the Examination Committee were as follows:

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Date: 27 November 2008

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

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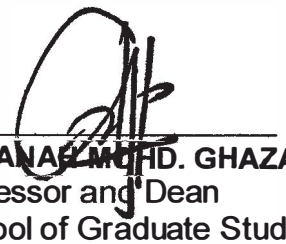

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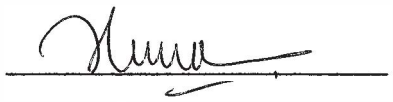
Date: 19 December 2008

DECLARATION

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



NORAZALINA SAAD

Date: 28.10.2008

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AEC	Anion-exchange chromatography
ACF	Aberrant crypt foci
AM	Azomethane
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
AOM	Azoxymethane
APC	Adenomatous polyposis coli
APES	3-aminopropyltrimethoxysilane
BERNAS	Padi Beras Nasional Berhad
BSA	Bovine Serum Albumin
°C	Degree celsius
Ca^{2+}	Calcium
COX	Cyclooxygenase
COX-2	Cyclooxygenase-2
CPA	Commercial phytic acid
DAB	3,3'-diaminobenzidine
DAG	Diacylglycerol
DMH	Dimethylhydrazine
DNA	Deoxyribonucleic acid
DPX	di-n-butylphthalate-polystyrene-xylene
EDTA	Ethylenediaminetetraacetic acid

EGF	Epidermal growth factor
EPA	Extract phytic acid
FAP	Familial adenomatous polyposis coli
FFA	Free fatty acids
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric ion
g	Gram
g/ml	Gram/mililiter
GSK3ß	Glycogen synthase kinase
H^+	Hydrogen ion
H & E	Hematoxylin and eosin
H_2O	Water
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HNPCC	Hereditary non-polyposis colorectal cancer
HPLC	High Performance Liquid Chromatography
hrs	Hours
IP3	Inositol triphosphate
IP4	Inositol tetraphosphate
IP5	Inositol pentaphosphate
IP6	Inositol hexakisphosphate
$InSP_3$	Inositol 1,4,5-trisphosphate
IL1	Interleukin-1

IL2	Interleukin-2
K^+	Kalium
Kcal	Kilocalorie
M	Molar
MAM	Methylazoxymethanol
MD	Methyldiazonium
Mg^{2+}	Magnesium ion
MHz	Megahertz
µg	Microgram
mg	Miligram
ml	Milliliter
mM	Milimolar
mm^2	Millimeter square
NaCl	Natrium chloride
NaOH	Natrium hydroxide
NSAIDs	Nonsteroidal anti-inflammatory drugs
OH^-	Hydroxide ion
O_2	Oxygen
PBS	Phosphate-buffered saline
PDGF	Platelet-derived growth factor
PCNA	Proliferating Cell Nuclear Antigen
PGI2	Prostacyclin
PGE2	Prostaglandin E2

PGD2	prostaglandin D2
PGH2	Prostaglandin H2
PGs	Prostaglandins
pH	Per hydrogen
RD	Rhabdomyosarcoma
SD	Standard deviation
SPSS	Statistical Package for the Social Science
TBS	Tris Base Saline
TBST	Tris-buffered saline with Tween-20
TCA	Trichloroacetic acid
Tcf	T cell factor
TNF	Tumor necrosis factor
TxA2	Thromboxane
UV	Ultraviolet
UVB	Vltraviolet light B
v/v	Volume/volume
WHO	World Health Organization
w/v	Weight/volume