UNIVERSITI PUTRA MALAYSIA

POTENTIAL OF GERMINATED BROWN RICE AND ITS BIOACTIVES AS REPLACEMENT FOR HORMONE THERAPY IN MENOPAUSAL MODEL

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By

ISMAILA MUHAMMAD SANI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

THIS THESIS IS DEDICATED TO MY PARENTS ALHAJI SAMAILA BABA, HAJIYA AISHA AND HAJIYA AMINA FOR THEIR LOVE AND CARE

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Febuary 2014

Chair: Prof. Maznah Bint Ismail, PhD

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Menopause is a stage in a woman's life when her menstrual cycle ceases due to the gradual decrease of sex steroid hormone estrogen in circulation. Mood swings, hot flashes, bone and heart diseases are among the characteristic features that affect women in menopause. Currently, hormonal therapy (HRT) has been employed in the management of patients with menopausal disorders; however, HRT is associated with carcinogenicity and other related health problems. Germinated brown rice (GBR) contains antioxidants and bioactive compounds. In this study, GBR bioactive compounds were evaluated independently as potential agents to replace hormone therapy. The antioxidant effects of the GBR- Phenolics using various antioxidant assays were carried out, while ovariectomized rats were used as a model in-vivo. Results for the GBR-Phenolics antioxidant values showed a significant increase ($p<0.05$) in antioxidant values and the total phenolic content(TPC) and DPPH results when comparing neutral with acidic and basic catalysed hydrolysis samples, yield was slightly higher in acidic hydrolysis than in basic hydrolysis ($p>0.05$), with TPC and TFC being highest in acidic hydrolysis. A significant correlation was observed between ABTS and FRAP. Antioxidant activity using DPPH was higher in acidic medium, while the ABTS % scavenging activity and FRAP showed the highest values in basic hydrolysis.

Bone density increases significantly ($p<0.05$) in rats treated with estrogen, GBR, remifemin and oryzanol compared to ovariectomized non-treated group. Histological section reveals more osteoblast in the treated groups compared with the un- treated groups; and a good correlation was obtained when results of bone densities obtained using Archimedes principle were compared with those obtained using the Edge detection technique between the treated groups. GBR and its bioactives, significantly increased the weight and length of both the uterus and the vagina than the OVX-non treated ($p<0.05$). Significant changes were observed in the ratio of cornified epithelial cells and number of leucocytes in the vaginal cytology between the oophrectomized and the treated groups. There was also an increase in the luminal and glandular epithelial cells activity in the treated compared with the un-treated groups histologically. Groups treated with GABA100 and 200 mg/kg showed significant up-

regulation of Sparc, Calcitonin and BMP-2 genes ($p<0.05$).While Oryzanol treated group at 200 and 100 mg/kg revealed significant ($p<0.05$) up-regulation of OSX, Postn, RUNX-2 and collagen 1&2. Similarly, IL-6 concentration decreased, while osteocalcin level increased significantly ($p<0.05$) in treated group as compared to ovariectomized non-treated groups. This study gives a clue in the management of menopause and associated post-menopausal metabolic complications using GBR and its related bioactive compounds.

Abstrak tesis yang dikemukakankepadaSenat Universiti Putra Malaysia sebagai memenuhi keper luan untuk ijazah Doktor Falsafah

POTENSI BERAS PERANG CAMBAH (GBR) DAN SEBATIAN BIOAKTIF NYA SEBAGAI PENGGANTI TERAPI HORMON (HRT) DALAM MODEL MENOPAUS

By

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Menopaus adalah peringkat dalam kehidupan wanita apabila kitaran haid terhenti disebabkan oleh penurunan beransur-ansur seks hormon steroid estrogen dalam edaran. Perubahan mood , hot flashes , tulang dan penyakit jantung adalah antara ciri-ciri sifat yang menjejaskan wanita dalam menopaus. Pada masa ini, terapi hormon (HRT) telah diambil kerja dalam pengurusan pesakit dengan gangguan menopaus , namun HRT dikaitkan dengan kekarsinogenan dan lain-lain masalah kesihatan yang berkaitan. Bercambah beras perang (GBR) mengandungi antioksidan dan sebatian bioaktif. Dalam kajian ini, GBR sebatian bioaktif telah dinilai secara bebas sebagai agen yang berpotensi untuk menggantikan terapi hormon. Kesan antioksidan GBR - phenolic menggunakan pelbagai ujian antioksidan telah dijalankan , manakala tikus ovariectomized telah digunakan sebagai model dalam vivo. Keputusan untuk nilai antioksidan GBR - phenolic menunjukkan peningkatan yang signifikan ($p < 0.05$) dalam nilai-nilai antioksidan dan jumlah kandungan fenolik (TPC) dan keputusan DPPH apabila membandingkan neutral dengan sampel hidrolisis pemangkin berasid dan asas, hasil adalah lebih tinggi sedikit pada hidrolisis berasid daripada dalam hidrolisis asas ($p> 0.05$) , dengan TPC dan TFC yang tertinggi dalam hidrolisis berasid. Hubungkait yang ketara diperhatikan antara ABTS dan FRAP . Aktiviti antioksidan menggunakan DPPH adalah lebih tinggi pada medium berasid, manakala % aktiviti memerangkap ABTS dan FRAP menunjukkan nilai tertinggi dalam hidrolisis asas.

Kepadatan tulang meningkat dengan ketara ($p < 0.05$) pada tikus yang dirawat dengan estrogen, GBR , remifemin dan oryzanol berbanding dengan kumpulan bukan dirawat ovariectomized . Seksyen histologi mendedahkan lebih osteoblast dalam kumpulan dirawat berbanding dengan kumpulan un dirawat dan korelasi yang baik telah diperolehi apabila keputusan ketumpatan tulang diperolehi dengan menggunakan prinsip Archimedes telah dibandingkan dengan yang diperoleh menggunakan teknik pengesanan Edge antara kumpulan-kumpulan yang dirawat. GBR dan bioactives dengan ketara meningkatkan berat badan dan panjang kedua-dua rahim dan faraj daripada OVX bukan dirawat ($p < 0.05$). Perubahan yang ketara dapat diperhatikan dalam nisbah sel-sel epitelium cornified dan bilangan leukosit dalam sitologi faraj antara oophrectomized dan kumpulan yang dirawat. Terdapat

juga peningkatan dalam aktiviti berongga dan sel-sel epitelium kelenjar dalam dirawat berbanding dengan un - dirawat kumpulan histologi . Kumpulan dirawat dengan GABA100 dan 200 mg / kg muncul peraturan - besar Sparc , calcitonin dan BMP -2 gen ($p < 0.05$). Walaupun oryzanol kumpulan dirawat di 200 dan 100 mg / kg mendedahkan yang signifikan ($p < 0.05$) up- peraturan daripada OSX, Postn , RUNX -2 dan kolagen 1 & 2. Begitu juga, IL -6 kepekatan menurun, manakala tahap osteocalcin meningkat dengan ketara ($p <0.05$) dalam kumpulan dirawat berbanding dengan kumpulan-kumpulan bukan dirawat ovariectomized.Kajian ini memberi satu petunjuk dalam pengurusan menopaus dan selepas yang berkaitan komplikasi metabolik menopaus menggunakan GBR dan sebatian berkaitan bioaktif.

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I certify that a Thesis Examination Committee has met on (17/02/2014) to conduct the final examination of Ismaila Muhammad Sani on his thesis entitled "Potentials of Germinated Brown rice and its Bioactives as Replacement to Hormone therapy in Menopausal Model" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Menopause simply means the cessation of menstrual circle, it involves climacteric disturbances or changes in normal physiological functions that occur in women by the age of 49-50years or earlier which happens due to low level of circulating estrogen triggered by the regression and atrophy of the ovaries (Greendale & Judd, 1993; Ward et al., 2009). The word was coined particularly for human females which describes the cessation of monthly menses. But it also occurs in some other animals, many of which do not have monthly menstruation where the term indicates an end to fertility. At the age of puberty, a woman releases an egg monthly for fertilization from the thousands of eggs stored in her ovaries and the uterus. If the egg is not fertilized, progesterone levels decreases and the uterine lining sheds and bleeds as menstruation. At later ages of about 40 years, ovaries began to regress producing less estrogen and progesterone leading to decrease number of eggs, this decrease in estrogen causes changes in various organs and tissues within the body which include the uterus, vulva, vagina, heart, blood vessels, breast, bladder, brain, skin and bones. Menopause is associated with symptoms among which include, changes in menstrual cycle, hot flashes, insomnia, vaginal dryness, memory and concentration problems, night sweat, fatigue, heavy bleeding, hair loss, depression, headache, sexual disinterests, mood swings, urinary incontinence, and weight gain (Lock, 2002).

The hormonal changes that occur in menopause are linked to free radicals damage in the form of oxidative stress. Menopause is associated with an increase in oxidative stress and a decrease in some antioxidants, such as ascorbic acid, α-tocopherol, total thiols and erythrocyte glutathione (Vural et al., 2005). The long term consequences of population aging are becoming a reality with only 45 men alive for every 100 women by the age of 85 years. The life span of the Malaysian woman has increased from 71.6 years in 1980 to 74 years in 1995 (Wong & Nur Liyana, 2007). That is to say one third of her life is now spent in the menopause. A recent survey on the incidence, morbidity and mortality due to diabetes in Malaysia was found to be more prominent in women in their menopausal ages (Letchuman et al., 2010), With increasing awareness and a desire to live a healthy life after menopause, many Malaysian women now make a voluntary attempt to attend menopause clinics.

Hormone replacement therapy (HRT) has been traditionally used in the management of menopausal symptoms, but most women are not willing to take it due to one reason or the other. It has been reported that HRT is associated with unwanted side effects like vaginal bleeding, bloating and depression; other side effects include cholethetiasis, breast tenderness, mood changes and various thromboembolism (Beral et al., 2002; Ross et al., 2000; Williams et al., 1994). In search for alternative to HRT due to these effects, several alternatives are explored in order to manage post menopausal complication. In the present condition, some of these alternative mimic estrogen (like soy isoflavones) while some of them are still under trial and screening for toxicity and some are scarce, localized to a region or very expensive. In view of these short comings, other alternative are still needed to convert post menopausal complications.

Previous researches shows that high intake of whole grain-containing diet provides numerous beneficial effects on cancer (Jacobs et al., 1998), cardiovascular diseases

(Anderson, 2003; Newby et al., 2003) and some other chronic diseases(Liu et al., 2000). Germinated brown rice is derived from brown rice by soaking in water at 25^0C and allowed to undergo germination process for some regulated hours, and germinated at least 1mm long (Kiing & Wong, 2009; Musa et al., 2011). Brown rice grain contains a lot of nutritional components such as γ- aminobutyric acid (GABA), γ- oryzanol, dietary fibers, phytic acid, and vitamin E(Imam et al., 2012; Kiing et al., 2009; Sawaddiwong et al., 2008). During germination, nutrients in the brown rice change drastically. Nutrients that increase in content include γ-amirobutyric acid (GABA), dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium,zinc, γ-oryzanol, and prolylendopeptidase inhibitor(Kayahara & Tukahara, 2000).

In this respect, germinated brown rice is known for its anti-colon cancer and neuroprotective effects (Ismail et al., 2012; Latifah et al., 2010). Recently, Imam et al., established that, germinated brown rice have the ability of regulating some xenobiotic genes and also up-regulating antioxidant statue in diabetic rats (Imam & Ismail, 2012; Imam et al., 2012).

The increase in the morbidity rate of cancer and other side effects that arises due to the use of estrogen and other synthetic selective estrogen receptor modulators stimulates researchers in identifying other sources of managing menopausal symptoms closer to an ideal selective estrogen receptor modulator.

In this study, we aimed at exploring some bioactive compounds from germinated brown rice, which is in abundant as a staple food to all corners of the world as possible alternative to HRT. This research will give a clue on the effect of these compounds in some selective organs that were affected due to low estrogenic level such as the uterus and the bone. Their effects on Bone mass density and the molecular expression genes related to bone formation and estrogen regulated genes in uterus will be considered.

General objective

The general objective of this study is to investigate the potentials of germinated brown rice and its bioactive compounds as a replacement to hormone therapy in menopouse.

Specific objective

1. To determine the effects of acidic and basic hydrolysis on the yield of GBR-Phenolics, and to extract and quantify GABA, ASG and Oryzanol, from GBR.
2. To determine the effects of GBR and its bioactives on vaginal and uterine tissues of ovariectomized rats.
3. To determine the genes expressed in relation to bone metabolism
4. To determine the effects of GBR and its bioactives on Bone Mass Density (BMD) in ovariectomized rats.
5. To determine the effects of GBR and its bioactives on Estrogen receptor β immunoreactivity, serum steroid hormone concentration and mRNA expression in the uterus of ovariectomized rats.

Statement of the problem and Justification

- Menopause is becoming more a topic of concern in Malaysia and the world in general.
- Hormone replacement therapy is associated with a lot of complications.
- The use of phyto-estrogens also mimics the unwanted effects observed in HRT.
- This necessitates the need to explore other compounds with higher antioxidant activities for its management.

Hypotheses of the study were:

1. **Null hypothesis (Ho):** Germinated brown rice and its bioactive can not ameliorate menopausal symptoms in ovariectomized rats model
2. **Alternative hypothesis (H_1):** Germinated brown rice and its bioactive can ameliorate menopausal symptoms in ovariectomized rats model

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Conclusion

Although GBR bioactives regulate the activity of some estrogen-induced genes in the uterus, and our immunohistochemical study showed positive expression of ER-β immunoreactivity in the uterus, studies are still needed to further characterize, and confirm the selective estrogenic effects of these bioactives by in situ hybridization, ER-α-specific binding, and other molecular techniques.

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