UNIVERSITI PUTRA MALAYSIA

EFFECTS OF OIL PALM LEAF EXTRACT IN ENHANCING MEMORY, IMPROVING BONE MASS, AND REDUCING ANXIETY IN OVARIECTOMISED RATS

AHDAB BAKHSH

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By

AHDAB BAKHSH

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

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Faculty: Bioscience

Long term estrogen deficiency in women often leads to decrease in cognitive function and bone density. This study reports on the in vivo mineral bone-density, anxiety and cognition enhancing effects of the supplementation with phytoestrogenic oil palm leaves extract (OPL) in normal and estrogen-deficient ovariectomized female Sprague Dawley rats which was compared to green tea (GT). The rats were divided into five experimental groups: (1) Normal rats (control N); (2) ovariectomized rats (OVX control); and OVX rats supplemented with either (3) green tea (2.0% w/v) [OVX+GT]; (4) 150 mg OPL/kg body weight (BW) [OVX+OPL (150)]; or (5) 300 mg OPL /kg BW [OVX+OPL (300)] in the drinking water. The Morris Water Maze (MWM) analysis was used to test cognition in the ovariectomized rats. Anxiety was determined by measuring corticosterone (CORT) and estradiol (E2) levels in the blood serum, as compared to the normal control group. Determination of CA1 pyramidal cells of the hippocampus was performed on the brain,
whereas the bone loss analysis was conducted by measuring the weight of femur and tibia calcium levels, together with histopathological observations.

The results of MWM showed the effects of long-term memory (LTM) and short-term memory (STM), were significantly different for all groups throughout the study. During the second and third months, the STM was significantly higher in the [OVX+OPL (300) and (150)], as well as OVX+GT. The OVX group took the longest time to reach the hidden platform indicating decreasing STM. The OVX+GT rats had the significantly highest level of LTM as compared to the other groups. The histopathology observation on the CA1 hippocampal pyramidal cells showed significant decrease in the OVX group as compared to the other groups. The serum CORT and E2 serum level were significantly different between the groups; with GT exhibiting the lowest CORT level during the three months followed by OVX+OPL (300) and (150). Meanwhile, the OVX group had the highest CORT level from the first to the second month. All the groups showed an increase in their E2 from zero to the third month. Finally, the phytoestrogenic OPL dose dependently enhanced OVX bone-density, calcium contents, ash weights and T-ALP to above even the normal rats and improved bone structure.

The findings from this study support the hypothesis that, the OPL dose dependently improved the cognitive functions and decreased anxiety of the estrogen deficient OVX rats towards normal control rats. The OPL dose dependently improved bone calcium density, significantly over the normal rats. The OPL treatments could prevent several maladies related to estrogen deficiency.
Key words: Oil Palm leaves, Green tea, Ovariectomy, cognitive function, Anxiety, Bone density.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master of Science

KESAN EKSTRAK DAUN KELAPA SAWIT DALAM MENINGKATKAN DAYA INGATAN, KETUMPATAN TULANG, DAN MERENDAHKAN KERUNSINGAN PADA TIKUS BETINA YANG KETIADAAN OVARI

Oleh

BAKHSH AHDAB

Jun 2012

Penurunan estrogen pada wanita yang telah menopause menyebabkan fungsi kognitif dan ketumpatan tulang berkurangan. Kajian in vivo ini melaporkan kesan ke atas ketumpatan tulang, kerunsingan dan kognisi, ekstrak daun kelapa sawit (OPL) yang fitoestrogenik pada tikus betina yang normal dan yang kekurangan estrogen selepas dibuang ovarinya apabila dibandingkan dengan teh hijau. Kesemua tikus telah dibahagikan kepada lima kumpulan ujikaji: (1) Tikus normal (Kawalan, N); (2) Tikus yang telah dibuang ovari (Kawalan OVX); dan tikus yang telah dibuang ovari (OVX) dan diberi minum sama ada (3) teh hijau (2.0% berat/isipadu) [OVX+GT]; (4) 150 mg ekstrak daun kelapa sawit/kg berat badan (BW) [OVX+OPL (150)]; atau (5) 300 mg ekstrak daun kelapa sawit/kg berat badan [OVX+OPL (300)] dalam air minuman. Analisis ‘Morris Water Maze’ (MWM) telah pun dibuat untuk menguji fungsi kognitif pada tikus-tikus yang telah dibuang ovarinya. Tahap kerunsingan telah diperiksa dengan mengukur hormon...
‘corticosterone’ (CORT) dan ‘estradiol’ (E2) dalam serum darah dan yang dibandingkan dengan kumpulan kawalan. Sementara itu, penentuan sel-sel CA1 Pyramid pada hipokampus pada otak telah diperiksa manakala analisis kehilangan ketumpatan tulang telah dijalankan dengan mengukur kandungan kalsium dalam femur dan tibia bersama-sama dengan pemerhatian histopatologi.

Keputusan-keputusan MWM telah pun menunjukkan kesan perbezaan yang ketara pada memori jangka panjang (LTM) dan memori jangka pendek (STM) terhadap semua kumpulan uji kaji dari permulaan sehingga bulan ketiga. Sepanjang bulan kedua dan ketiga, perbezaan STM yang ketara dalam ketiga-tiga kumpulan [OVX+OPL (300) dan (150), dan OVX+GT). Kumpulan OVX mengambil masa yang paling lama untuk sampai ke platform yang tersembunyi. Sebaliknya, kumpulan OVX+GT) menunjukkan LTM yang tertinggi apabila dibandingkan dengan kumpulan-kumpulan lain. Keputusan MWM telah disahkan selepas pemerhatian histopatologi pada sel-sel CA1 Pyramid hipokampus kerana ia menunjukkan penurunan sel-sel CA1 dalam kumpulan OVX apabila dibandingkan dengan OVX+GT dan kumpulan normal, kawalan. Tambahan pula, analisis pada tahap serum CORT dan E2 menunjukkan perbezaan yang ketara antara kesemua kumpulan kerana GT mempamerkan penurunan dalam CORT sepanjang tiga bulan berturut-turut diikuti dengan kumpulan OVX+OPL (300) dan (150). Sementara itu, kumpulan OVX menunjukkan peningkatan tertinggi tahap CORT dari bulan pertama ke bulan kedua. Kesemua kumpulan menunjukkan peningkatan dalam E2 dari permulaan ke bulan ketiga. Akhir sekali, keputusan femur dan tibia dari kumpulan OVX+OPL (150) dan (300) menunjukkan jumlah kalsium yang tertinggi apabila dibandingkan dengan kumpulan-kumpulan lain. Berat badan menunjukkan perubahan yang ketara pada
kesemua kumpulan, manakala kumpulan OVX+GT menunjukkan berat badan yang paling rendah diikuti kumpulan OVX+OPL (300) dan (150) berbanding kumpulan kawalan, normal. Kumpulan OVX tanpa rawatan mempamerkan berat badan yang tertinggi.

Penemuan-penemuan daripada kajian ini menyokong hipotesis OPL dengan kepekatan yang tinggi (300) dan rendah (150) menunjukkan peningkatan dalam keupayaan kognitif manakala ia juga menurunkan tahap kerunsingan apabila dibandingkan dengan kumpulan OVX dan kumpulan normal, kawalan. Walau bagaimanapun, hal ini tidak ketara dalam kumpulan GT yang menunjukkan peningkatan yang hebat. Tambahan pula, kedua-dua kepekatan OPL meningkatkan ketumpatan kalsium tulang dengan perbezaan ketara dari kumpulan-kumpulan lain. Kumpulan rawatan OPL boleh mencegah beberapa ‘maladies’ yang dikaitkan dengan kekurangan estrogen.
ACKNOWLEDGEMENTS

At the beginning, all praise due to Allah, lord of the world. Only by his Grace and Mercy this work has been finished and incha Allah it will be successful.

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I certify that a thesis examination committee has met on……………. 2012 to conduct the final examination of Ahdab Bakhsh on his thesis entitled ´´Application of Oil Palm Leaves Extract in Enhancing Memory and Bone Improvement, and Reducing Anxiety Under Estrogen Deficiencyon Female Rats `´ in accordance with the Universities College 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 Master of Science.

Members of the Thesis Examination Committee were as follow:

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

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Professor and Dean
School of graduate Studies
Universiti Putra Malaysia

Date:
DECLARATION

I declare that the thesis is 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.

BAKHSH AHDAB

Date: 01 June 2012
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<td>Bone mineral density</td>
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<td>ROS</td>
<td>Reactive oxygen spaces</td>
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<td>GT</td>
<td>Green tea</td>
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<td>EGCG</td>
<td>Epigallocatechin gallate</td>
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<td>OPL</td>
<td>Oil palm leaves extract</td>
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<td>LDL</td>
<td>Low density lipoprotein</td>
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STM          Short term memory
LTM          Long term memory
Control N    Control normal
OVX          Ovariectomized
OVX+OPL      Ovariectomized treated with oil palm leaves
OVX+GT       Ovariectomized treated with green tea
NMDA         Nmethyl D- sparate
T-ALP        Total alkaine phosphatase
Ca           Calcium
P            Phosphorus
Ng           Nanogram
Mg           Milligram
Sec          Second
μm           Microgram
Cm           Centimeter
Mm           Millimeter
G            Gram
mmol/L       Milimole per liter
U/l          Units per liter
U/mg         Units per mile gram
pg/ml  Pictogram per milliliter
i.e  Example
FAE  Formic Acid equivalent
µg/g  Micro gram per gram
HRT  Hormonal replacement therapy
AD  Alzheimer disease
BM  Bone mass
AAS  Atomic Absorbance Spectrophotometry
CHAPTER 1

GENERAL INTRODUCTION

A woman is considered to be post-menopausal when her menstrual cycles stop, indicating that her ovaries have stopped producing sex hormones (progesterone and estrogen) or after removal of her ovaries. Even though every woman will eventually experience menopause, the experience for every individual may be different, and there are some cases where women go for medical consultation on ways to manage the symptoms (Foth, 2010). During menopause, the rate of brain processing is delayed by reduced production of estrogen. Furthermore, postural stability is significantly affected by this change, as it depended on identification of sensory input and commencement of suitable physical response. Notably, the rate of women involved in fall-injuries is three times higher compared to men. Bone density and postural stability are closely related to contributing to fractures in women suffering from osteoporosis. Estrogen is proven to improve bone density and postural stability. Oestrogen’s ability to prevent non-vertebral fall-related fractures is demonstrated better compared to alendronate and raloxifene (Foth, 2010).

The cognitive functioning in women is also affected by the production of endogenous estrogens. Nelson, (2008) inferred that the action of estradiol in assisting cognitive functions, such as short-term memory, learning, and attention, correlate with the genomic estrogenic effects in which it responds as a growth factor in the formation of new synapses by estrogen-responsive dendritic neurons. Moreover, the increased
production of stress hormone cortisol and activation of hypothalamic-pituitary axis caused by chronic stress is perceived to enhance the rate of brain aging. Memory is enhanced if the brain is exposed to stress for a short period; however, long-term exposure to stress has been shown to result in a permanent damage of the hippocampus, which is the brain region responsible for declarative memory (Nelson, 2008). Hence, estrogen is also neuroprotective.

For older individuals who are suffering from Alzheimer’s disease (AD), the risk of fractures is higher compared to those who are cognitively healthy (reference). In addition, once a hip fracture occurs, those with AD have lower capacity to recover and have higher mortality rates (Buchner & Larson, 1987; Weller & Schatzker, 2004). Tan et al., (2005) highlighted that there were studies that proposed the correlation between low bone mineral density (BMD) with higher risk toward getting AD and cognitive decline (Lui et al., 2003; Yaffe et al., 1999). However, there is little information on bone health in individuals suffering from AD.

There is evidence showing that bone health is mainly controlled by the central nervous system, especially by the hypothalamus (Harada & Rodan, 2003; Zaidi, 2007), in which this region of the brain is highly influenced by AD (Callen et al., 2001; Raskind et al., 1999). Nevertheless, the assessment on bone health affecting cognition and brain structure has never been done before. There were studies that showed post-menopausal estrogen replacement therapy (ERT) (Henderson et al., 2000; Shumaker et al., 2003) might have positive effects on cognition in both post-menopausal and pre-menopausal women (Barrett-Connor & Kritz-Silverstein, 1993; Yaffe et al., 1998). Some studies
conducted to observe the correlation between ERT and possibility of getting dementia had shown that the women who received ERT were better educated, physically healthier, and generally more compliant to medications compared to those non-users (Egeland et al., 1991; Petitti, 1994).

Many women who have been receiving ERT for several years are not capable of remembering the period of intake. The effect of endogenous estrogen is varied among women due to these factors: menopause, oophorectomy, and different age at menarche. Apart from these, there are also other factors that contribute to the difficulty in estimating the long-term effects of taking estrogens, both exogenous and endogenous. For collective exposure to estrogen, BMD could be used as a surrogate marker. Fox et al. (1993) reported that bone mass (BM) is directly associated with early menarche, late menopause, and parity. Nguyen et al. (1996) added that collective contact to endogenous and exogenous estrogens is also correlated to BMD. On the other hand, a report by Behl, (2002) highlighted that the phenolic compound in phytoestrogen structurally resembles α-tocopherol and a lipophilic antioxidant, which is capable of neutralizing reactive oxygen species (ROS).

Post-menopausal women have been using hormone replacement therapy, such as progestagens and oestrogens, to alleviate the changes they experience, including behavioral and physiological changes (Kotz et al., 2006; Nelson, 2008). These changes can cause mood swings, irritations, hot flashes, and bone loss (Freedman, 2002). The use of hormone therapy, however, is limited due to the side effects. Phytoestrogens have been shown to mimic oestrogens and react with the oestradiol receptor-β (Farmakalidis
et al., 1985; Kuiper et al., 1997; Markiewicz et al., 1993; Song et al., 1999). An anxiolytic-like effect of oestrogens has been shown by the receptor in laboratory tests (Lund et al., 2005; Walf & Frye, 2005; Weiser et al., 2008). Moreover, according to Cornwellet al. (2004), some compounds may not exert estrogenic effect until they are consumed, for example, lignans, which can be confusing for classification. It is noted that there are more than 300 plants having this characteristic, whereby different plants have different phytoestrogenic effects.

Green tea (GT), which is a popular drink, is made from the leaves of *Camellia sinesis* that contains polyphenols, functioning as antioxidants that eliminate ROS (Siddiqui et al., 2004). Research on cardiovascular diseases and cancer preventions by GT is extensive (Zaveri, 2006). Notably, the most active and prevalent compound of GT catechins is epigallocatechingallate (EGCG), which reduces peroxidation of lipids by inhibiting the free radicals (Manolagas, 2008; Yalin et al., 2006). These studies showed that bone loss and degenerated brain functions caused by estrogen deficiency can be alleviated by the antioxidant properties of green tea polyphenols (Sontakke & Tare, 2002).

In the oil palm industry, the leaves of the oil palm are the major waste products, which observed to have high amount of catechins and flavonoids. It could have comparative antioxidant activity to other plant and it was marked to possess higher antioxidant activity than GT. In order to get benefits from the waste of the production of OPL, the present study was used OPL as phytoestrogen replacement rather than other edible plants.
In accordance with Runnieet al. (2003), the gallic acid equivalent (GAE) per gram of the dry weight methanol extract of OPL is 24.3 mg, which is higher than GT, with 22.5 mg of GAE per gram of the dry weight. Most of the phenolic compounds in OPL, namely, catechins, carotenoids, and glycosylated flavonoids, have not been widely used as replacement treatments to prevent bone loss and degenerative brain disease. A study showed that the low density lipoprotein (LDL) oxidation had been better reduced by the OPL than other plant extracts (Salleh et al., 2002). In another study, the concentration of water soluble antioxidant compounds in the leaves also varied because the dried leaves showed higher activity than the wet counterpart (Han, May, 2010).

It appears that the number of study on the therapeutic features of OPL is still limited. Therefore, this study will provide some information on the oxidative properties of OPL in preventing the loss of bone density and cognitive functions. The assessment of OPL was questionable to have capability in treating cognitive function decline, anxiety and enhancing BMD. For this purpose, the main aim of this study was to determine the effect of two doses of OPL, 300 mg/kg/day and 150 mg/kg/day, which were selected from the used range by several researchers. They were dissolved in water before being given to the rats and their effect were compared to 2% GT, which is equal to 4 cups/day of human consumption on the cognitive functions, anxiety, and bone health in estrogen-deficient mammalian model.
The objectives of the study were as follows:

1. To study the effect of OPL extracts of different doses in comparison to GT on cognitive ability in ovariectomized estrogen-deficient rats.
2. To study the effect of OPL extracts of different doses in comparison to GT on anxiety in ovariectomized estrogen-deficient rats.
3. To study the effect of OPL extracts of different doses in comparison to GT on bone density in ovariectomized estrogen-deficient rats.
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BIODATA OF STUDENT

BAkhsh Ahdab Abdulrazzaq was born on 29<sup>th</sup> April 1985 in Makkah, Saudi Arabia. She did her primary, secondary and high schools in her home town. He moved to Um Al-Kurah University for her bachelor degree in Food science and she graduated on 2007. She registered as postgraduate student in UPM at the faculty of food science and technology under food science field on December 2009. She transferred to institute of bioscience under the field of phytochemistry.
LIST OF PUBLICATIONS


SEMINARS


2. Rat: Basic Handling and Technique Workshop, 2011 , UPM university , (organiser)