Serum levels of 17β -estradiol in ovariectomized rats fed young-coconut-juice and its effect on wound healing

Nisaudah Radenahmad¹, Uraporn Vongvatcharanon², Boonsirm Withyachumnarnkul³ and James R. Connor⁴

Abstract

Radenahmad, N., Vongvatcharanon, U., Withyachumnarnkul, B. and Connor, J.R. Serum levels of $17\beta\text{-estradiol}$ in ovariectomized rats fed young-coconut-juice and its effect on wound healing

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Exogenous estrogens such as 17β -estradiol (E2) can lower the risk of dementia in postmenopausal women (PMS), but can also increase the risk of serious side effects. Estrogen can, however, promote wound healing in ovariectomized rats and in PMS women. Phytoestrogen (PE) derived from plants might be a safer alternative source of estrogen for use in hormone replacement therapy (HRT). Young coconut juice (YCJ), *Cocos nucifera* (Arecaceae), that is believed to contain PE and other sex hormone-like substances, was therefore investigated for its possible beneficial effects on accelerating wound healing in ovariectomized rats, a model system for the postmenopausal condition. Sixty ovariectomized (ovx) rats were devided into 6 groups, 10 rats per group. group 1 received E2 (i.p.) at 2.5 μ g/kgBW/day (control); groups 2 and 3 received YCJ at 20 mL and 100mL/kg BW/day, respectively. Group 4 received YCJ 100 mL/kg BW plus E2 at 2.5 μ g/kg BW/day twice a week, all for 5 weeks. The other two groups were ovx and sham-operated controls receiving

¹Ph.D.(Neuroscience), ²Ph.D. (Cardiology), Asst. Prof., Department of Anatomy, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand. ³Ph.D.(Neuroscience), Prof., Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, 10400 Thailand. ⁴Ph.D.(Anatomy), Prof., Department of Neuroscience and Anatomy, The Penn State University College of Medicine, P.O. Box 850, 500 University Drive Hershey, PA, 17033-0850, USA.

Corresponding e-mail: nisaudah.r@psu.ac.th

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vehicle, Milli Q water, like the rest, everyday once a day. Using a chemiluminescent immuno assay, circulating E2 in the ovariectomized group fed with YCJ at 100 mL/kg BW/day was not significantly different from the control group. Circulating E2 was lowest in the ovariectomized rats fed with 100 mL/kg BW of YCJ plus 2.5 μ g/kg BW E2 twice a week. This finding indicates an antagonist effect of estrogen-like hormones in YCJ that competes with estradiol for the estrogen receptors. In these experiments, we noted that wound healing was significantly accelerated in ovariectomized rats receiving 100 mL YCJ/kg BW/day compared with any other groups.

Key words: *Cocos nucifera*, wound healing, coconut juice, estrogen, postmenopausal women

บทคัดย่อ

นิซาอูดะห์ ระเด่นอาหมัด¹ อุราพร วงศ์วัชรานนท์¹ บุญเสริม วิทยชำนาญกุล² และ James R. Connor³ ระดับของ 17β-estradiol ในเลือดของหนูที่ถูกตัดรังไข่ออก และได้รับน้ำมะพร้าวอ่อน และผลต่อการสมานแผล

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การให้เอสโตรเจนเข้าไปในร่างกาย (exogenous estrogen) เช่น 17β-estradiol (E2) ช่วยลดโอกาสเสี่ยงของ การเกิดโรคความจำเสื่อมในสตรีวัยหมดประจำเดือน แต่ในขณะเดียวกันก็เพิ่มความเสี่ยงของผลข้างเคียงร้ายแรง ต่าง ๆ ด้วย อย่างไรก็ตาม ในหนที่ถกตัดรังไข่และในสตรีวัยหมดประจำเดือน เอสโตรเจนมีผลทำให้แผลหายเร็วขึ้น ไฟโตเอสโตรเจน (phytoestrogen) จากพืชจึงอาจเป็นฮอร์โมนทดแทนที่ปลอดภัยกว่าในการรักษาแบบ hormone replacement therapy (HRT) การวิจัยในครั้งนี้ได้ทำการศึกษาผลของน้ำมะพร้าวอ่อน Cocos nucifera (Arecaceae) ซึ่งประกอบด้วย ไฟโตเอสโตรเจน และฮอร์โมนเพศอื่นๆ ต่อหนูที่ทำการตัดเอารังไข่ออก ซึ่งเปรียบได้กับสตรีวัย หมดประจำเดือน ในการทำให้แผลหายเร็วขึ้น โดยแบ่งหนูที่ถูกตัดเอารังไข่ออก (ovx) 60 ตัวออกเป็น 6 กลุ่ม กลุ่ม ละ 10 ตัว กลุ่มที่ 1 คือกลุ่มควบคุมได้รับ E2 2.5 μ g/kg BW/day โดยฉีดเข้าทางหน้าท้อง (i.p.) กลุ่มที่ 2 และ 3 ได้รับน้ำมะพร้าวอ่อน 20 มล. และ 100 มล./กก./วัน กลุ่มที่ 4 ได้รับน้ำมะพร้าวอ่อน 100 มล./กก./วัน พร้อมทั้งฉีด E2 (i.p.) 2.5 ไมโครกรัม/กก./วัน สัปดาห์ละ 2 ครั้ง เป็นเวลา 5 สัปดาห์ สำหรับกลุ่มควบคุมอีก 2 กลุ่มคือกลุ่ม ovx และ sham-operated ได้รับกระสายยา (vehicle) คือ Milli Q water ทุกวัน วันละ 1 ครั้ง เช่นเดียวกับอีก 4 กลุ่ม ดังกล่าวข้างต้น จากการตรวจเลือดหนูด้วยวิธี chemiluminescent immuno assay พบว่ากลุ่มหนูที่ตัดเอารังไข่ออก และได้รับน้ำมะพร้าวอ่อน 100 มล/กก/วัน มีค่า E2 serum ไม่แตกต่างจากกลุ่มควบคุม และ E2 serum มีค่าต่ำสุด ในหนูกลุ่มที่ได้รับน้ำมะพร้าวอ่อน 100 มล./กก./วัน พร้อมทั้งฉีด E2 2.5 ไมโครกรัม/กก./วัน สัปดาห์ละ 2 ครั้ง การ ทดลองนี้แสดงให้เห็นว่าสารคล้ายฮอร์โมน เอสโตรเจน ในน้ำมะพร้าวอ่อนมีฤทธิ์ antagonist effect โดยแย่งจับกับ ตัวรับเอสโตรเจน (estrogen receptor) นอกจากนี้ยังพบว่าหนูที่ได้รับน้ำมะพร้าวอ่อน 100 มล./กก./วัน มีอัตราการ หายของแผลเร็วกว่าหนูกลุ่มอื่น ๆ อย่างชัดเจน

¹ภาควิชากายวิภาคศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112 ²ภาควิชากาย-วิภาคศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล ถนนพระราม 6 กรุงเทพฯ 10400 ³Department of Neuroscience and Anatomy, The Penn State University College of Medicine, P.O. Box 850, 500 University Drive Hershey, PA, 17033-0850, USA.

After menopause, estrogen levels decrease dramatically in women. This decrease in endogenous estrogen is associated with an increased risk of cardiovascular disease and osteoporosis (Christiansen and Lindsay, 1990). Decreasing estrogen levels are also claimed to be associated with an increased risk of dementia. A relationship between estrogen levels and dementia is biologic-

ally plausible (McEwen and Alves, 1999; Solerte et al., 1999; Gibbs and Aggarwal, 1998) and, if proven, may have major implications for the prevention or treatment of dementia. In ovariectomized (ovx) rats, estrogen injections increase choline acetyltransferase activity in the basal forebrain and hippocampus regions of the brain. These regions are acetylcholine deficient in patients with Alzheimer's disease (AD) (Gibbs and Aggarwal, 1998). In addition, estrogen improves synapse formation on dendritic spines in the hippocampus of oophorectomized rats (Monk and Brodaty, 2000; McEwen and Alves, 1999). Estrogen also may improve cerebral blood flow and glucose metabolism, and it may in some way act as an antioxidant (Monk and Brodaty, 2000; McEwen and Alves, 1999; Gibbs and Aggarwal, 1998; Birge, 1996). Another mechanism by which estrogen may exert an influence on the development of dementia is by reducing the risk of cardiovascular disease (Yaffe et al., 1998; Breteler et al., 1994). Many studies have reported a lower prevalence and incidence of dementia and AD in postmenopausal (PMS) women who used estrogen replacement therapy, suggesting that exogenous estrogen use may reduce the risk of dementia (Waring et al., 1999, Paganini-Hill and Henderson, 1994, 1996). Other studies have found that higher concentration of bioavailable estradiol was associated with a slower cognitive decline (Yaffe et al., 2000).

Although exogenous estrogen could reduce the risk of dementia in PMS women, it could also increase the risk of breast and ovarian cancer, as well as causing other side effects. Phytoestrogen derived from plants might be a viable alternative source for hormone replacement therapy (HRT) agents. According to folk medicine, coconut juice (*Cocos nucifera* L., Arecaceae), contains many active compounds that may have different therapeutic properties. For centuries, coconut juice has been used as a "temporary contraceptive" drink for both Thai and Indonesian people (Laszlo and Henshaw, 1954; Roig and Mesa, 1945, Brondegaard, 1973; Perry, 1980). It is said that people in Java are afraid to drink coconut milk as it is

believed to diminish fertility (Laszlo and Henshaw, 1954). With this background, young coconut juice (YCJ), presumably containing phytoestrogen, was investigated for its possible beneficial effect on accelerating wound healing in ovx rats, a model for postmenopausal women. Investigations are continuing on the possibility that YCJ can be used to halt Alzheimer's disease.

Materials and Methods

Animals

All animals used were adult 16 weeks female Wistar rats weighing approximately 270 g.The animals were housed in a controlled environment at 25±1°C on an illumination schedule of 12L:12D. Rats had access to standard pellet food and water *ad libitum*. The animal studies were approved by the Ethics Committee on Animal Care and carried out in accordance with the Guiding Principles for the Care and Use of Research Animals promulgated by Prince of Songkla University.

Experimental design

Two phases of preliminary study were carried out. This established the dose of 17β -estradiol (E2) required for injection into ovx rats to return levels to those found in a sham-operated group (6 rats/group). See the details below in "Titration of hormone".

Throughout this study, exogenous 17β -estradiol (E2) used was estradiol benzoate (EB).

The phase III study was performed as follows:

The 3 estrogen replacement groups of ovariectomized rats (10 rats per group) were daily force fed orally with either 20 mL, or 100 mL of reconstituted YCJ/kg BW/day or 100 mL YCJ/kg BW/day plus an E2 (i.p. injection) at 2.5 μ g/kg BW/day twice a week. Controls consisted of 3 subgroups (10 rats per group): ovx only, shamoperated and ovx injected with EB at 2.5 μ g/kg BW/day for 5 weeks. All animal groups and treatments are summarized as Table 1.

After the end of the experiment, rats were

Table 1. Treatment description for animal groups

Animal groups & symbols	Treatment		
OVX	Milli Q water* orally fed once everyday, 5 weeks		
sham	Milli Q water* orally fed once everyday, 5 weeks		
control (ovx + EB)	EB i.p. injection, 2.5 μg/kg BW/day, 5 weeks post-ovx@		
20 mL (ovx + YCJ 20 mL)	20 mL/kg BW/day YCJ feeding, 5 weeks post-ovx@		
100 mL (ovx + YCJ 100 mL)	100 mL/kg BW/day YCJ feeding, 5 weeks post-ovx@		
100 mL + EB (ovx + YCJ 100 mL + EB)	100 mL/kg BW/day YCJ feeding plus 2.5 μ g/kg BW/day EB i.p. injection, twice a week; 5 weeks post-ovx @		

^{*}Milli Q water was the solvent used to dissolve YCJ from freeze dried powder.

@ all animals were left 1 week after ovx before starting EB injections or YCJ feeding.

killed and serum was collected for estradiol measurements using a chemiluminescent immuno assay (CIA) kit (LKE2 10261, DPC, Gwynedd, UK).

Ovariectomy

Rats at 16 weeks of age were anesthesized with pentobarbital sodium (35 mg/kg BW, i.p.) and both ovaries were excised. Two-centimeter vertical skin incisions were made bilaterally on the sides of the abdomen at the mid-axillary line, 1.5 cm below the lower edge of the costal cage. A 1.5 cm vertical cut was then made in the subcutaneous tissue to expose the ovaries intraperitoneally. The ovaries were clamped and removed and the uterine tubes were ligated. The muscle and skin were then sutured. The sham procedure consisted of anesthesia, visualization of the ovaries through incisions into the abdominal cavity, and closure of the wounds. No antibiotic or any other medication was applied to the wounds during the following 6 weeks. Animals were housed for 7 days after ovariectomy before initiation of hormone treatment or YCJ feedings.

Titration of hormone

Estradiol benzoate (EB) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

EB is widely used as exogenous 17β -estradiol (E2). The hormone was dissolved in olive oil. To establish the dose of EB injected into ovx rats that would return levels to those found in a shamoperated group, 2 phases of preliminary study were carried out as follows:

Phase I. Four-month female rats were ovariectomized and left untreated for a week before starting subcutaneous injections of EB for one week at 5 μ g, 10 μ g, 50 μ g, and 100 μ g/kgBW/day.

Phase II. Four-month female rats were ovariectomized and left untreated for a week before starting subcutaneous injections of E2 for one week at either 2.5 μ g or 5 μ g/kgBW/day.

One week after injections ceased, rats were sacrificed and their uterus was removed and weighed.

Uterine weights expressed as a percentage of body weight in ovx rats after EB injections were compared between controls (ovx and shamoperated) and experimental groups (ovx + EB 2.5 and 5 μ g/kgBW/day). The uterine weight was used as an indicator for adjusting the optimal dose of E2 to inject into ovx rats to return to levels that were not significantly different from those in the sham-operated group.

Determination of circulating E2

On the terminating day, 2 weeks (phase I and II) and 6 weeks (phase III) after ovariectomy, rats were sacrificed, serum was collected and estradiol measurements using a chemiluminescent immuno assay (CIA) kit (LKE2 10261, DPC, Gwynedd, UK) were analysed.

Preparation of Young Coconut Juice

Young coconut juice was collected from 6 month old fruit from one area (100 square yards) in Namnoi district, Hat Yai, Songkhla, throughout this study. The young coconut juice was freeze dried (100 mL produced 5.5 g powder) and kept at -30°C until used. The powder was dissolved in Milli Q water to make up appropriate concentrations in order to feed each rat at 1 mL/100 g body weight. For example, a 250 g rat was fed with only 2.5 mL of reconstituted YCJ each day. This meant that each 2.5 mL contained either 0.275 g or 1.375 g of powder for a 250 g rat requiring either 20 mL YCJ/kg BW/day or 100 mL YCJ/kg BW/day respectively.

Statistical analysis

All results were represented as arithmetic means \pm SD. Non-parametric data were analyzed

with the Kuskal-Wallis analysis of variance (ANOVA) followed by the Mann-Whitney U-test for multiple comparisons between groups.

Results

Titration of E2

From the phase I experiment, EB injected at 10, 50 and 100 μ g/kg BW/day to ovx rats once everyday, at the same time, for 7 days resulted in extremely high E2 serum levels (2,431.66±1,563; 4,070±3,026.59 and 3,148.96±2,100 pg/mL respectively) at p<0.05. A sham control group had levels of E2 of 49.31±1.00 pg/mL. An EB injection at 5 μ g/kg BW/day also produced an E2 serum level (63.83±16.00 pg/mL) that was not significantly different from the sham-operated group and the ovx group (27.43±21.81 pg/mL) (Figure 1).

From the phase II experiment, a serum level of E2 (65.47 \pm 19.88 pg/mL) resulting from an EB injection into ovx rats at 2.5 μ g/kg BW/day was not significantly different from the sham-operated group (44.50 \pm 35.04 pg/mL) (Figure 2). In contrast, the E2 serum levels in the ovx group (16.12 \pm 1.36 pg/mL) and in ovx rats injected with EB 5 μ g/kg BW/day (77.95 \pm 16.98 pg/mL) showed a significant difference compared to the sham-operated

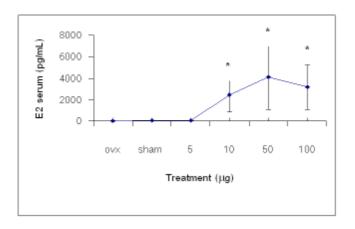


Figure 1. E2 serum levels (mean \pm SD) measured by chemiluminescent immuno assay (CIA) after estradiol benzoate (EB) injections (5, 10, 50, 100 µg/kg BW/day) to ovx rats once everyday, for 7 days compared to a sham control group. $5 = \text{ovx} + \text{EB} 5 \mu\text{g/kg}$ BW/day, $10 = \text{ovx} + \text{EB} 10 \mu\text{g/kg}$ BW/day, $50 = \text{ovx} + \text{EB} 50 \mu\text{g/kg}$ BW/day, $100 = \text{ovx} + \text{EB} 100 \mu\text{g/kg}$ BW/day. (* p<0.05).

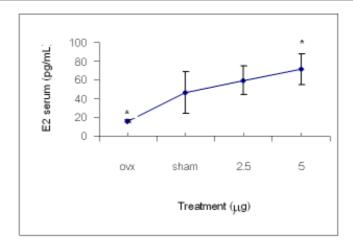


Figure 2. E2 serum levels (mean \pm SD) measured by chemiluminescent immuno assay (CIA) after estradiol benzoate (EB) injections (2.5, 5 μ g/kg BW/day) to ovx rats once everyday, for 7 days compared to a sham control group. 2.5 = ovx + EB 2.5 μ g/kg BW/day, 5 = ovx + EB 5 μ g/kg BW/day. (* p<0.05).

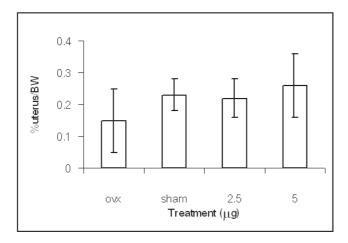


Figure 3. Weights of uterus expressed as a percentage of body weight (mean \pm SD) in ovx rats after estradiol benzoate injections (2.5, 5 μ g/kg BW/day) once everyday, for 7 days compared to the sham control and ovx groups. 2.5 = ovx + EB 2.5 μ g/kg BW/day, 5 = ovx + EB 5 μ g/kg BW/day.

group (p<0.05). All values of both phase I and II are expressed as mean \pm SD.

In addition weights of the uterus expressed as a percentage of body weight (mean \pm SD) in ovx rats after EB injections at 2.5 and 5 μ g/kg BW/day were not significantly different from those of the sham-operated and ovx groups (p>0.05) (Figure 3).

Level of E2 serum in YCJ

As shown in Figure 4, E2 serum levels assayed by CIA in ovx rats fed with YCJ at 100 mL/kg BW/day (65.67±8.51 pg/mL) were not significantly different from the sham-operated (46.13±25.05 pg/mL) or control (57.57±19.26 pg/mL) groups (p>0.05). Those of the sham and control groups were significantly different from

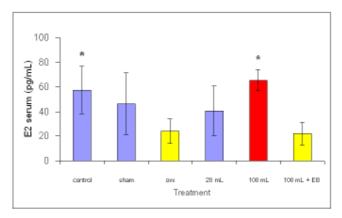


Figure 4. E2 serum levels (mean \pm SD) assayed by CIA. Control = ovx + EB 2.5 μ g/ kg BW/day; 20 mL = ovx + YCJ feeding 20 mL/kg BW/day; 100 mL = ovx + YCJ feeding 100 mL/kg BW/day; 100 mL+EB = ovx + YCJ feeding 100 mL/kg BW/day plus E2 2.5 μ g/kg BW injections twice a week. EB = estradiol benzoate. (*p<0.05 compared with ovx group).

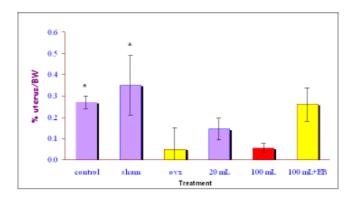


Figure 5. Relationship between the weight of the uterus expressed as a percentage of body weight (mean \pm SD) compared among control groups and ovx rats fed with young coconut juice (YCJ) for 6 weeks. Control = ovx + E2 2.5 $\mu g/kg$ BW/day; 20 mL = ovx + YCJ feeding 20 mL/kg BW/day; 100 mL = ovx + YCJ feeding 100 mL/kg BW/day; 100mL+EB = ovx + YCJ feeding 100 mL/kg BW/day plus EB 2.5 $\mu g/kg$ BW twice a week. EB = estradiol benzoate. (*p< 0.05 compared with ovx group).

that of the ovx group (24.13±9.75 pg/mL). E2 serum levels measured by CIA in ovx rats fed with YCJ at 20 mL/kgBW/day, although not significantly different (p>0.05) from the sham or ovx groups, did have a considerably lower level (40.67 ±19.86 pg/mL). Furthermore, E2 serum level in ovx rats fed with YCJ 100 mL/kg BW/day plus EB 2.5 µg/kg BW/day twice a week was much lower (22.20±9.27 pg/mL) than the control, sham and 100 mL/kgBW/day YCJ groups and, surprisingly, was not significantly different from the ovx group

(24.13±9.75 pg/mL) (p>0.05).

% Uterus/body weight

As shown in Figure 5, the weights of the uterus expressed as a percentage of body weight in ovx rats fed with either YCJ 20 mL/kg BW/day or 100 mL/kg BW/day (0.15±0.05, 0.06±0.02 respectively) were much reduced compared to the control (0.27±0.03), and sham (0.35±0.14) groups (p>0.05), whereas the weight of the 100 mL/kg BW/day plus EB 2.5 μg/kg BW/day (0.26±0.08)

was similar to those of the control and sham groups. The weights of the control and sham groups were significantly different from that of the ovx group (0.05 ± 0.01) (p<0.05).

Wound healing effect of YCJ

All photographs of the wounds in Figures 6-9 were taken from the worst wounds in each group. It is obvious that all wounds on the lateral sides of the ovx rats fed with 100 mL YCJ/kg BW/ day had the fastest healing rate e.g. less reddish, less swelling, compared to the other groups especially when compared to the ovx and control groups (e.g. more exudate, more swelling, more reddish). It took a shorter time, maximum of 11 days in the worst animals for wound closure in the ovx rats fed with 100 mL YCJ/kgBW/day while the ovx and control groups took as long as 21 days in the worst cases for the wound to close. Furthermore, at the end of the experiment, 6 weeks after ovx and the skin was shaved, in all rats fed with YCJ either 20 mL, 100 mL or 100 mL/kg BW/day plus EB 2.5 µg/kg BW/day, there was no scar left at the site of the skin cut. In contrast, in ovx, sham

and control groups, there was a yellow line scar left at the site of the skin cut for ovariectomy (Table 2).

Discussion

Injection of 2.5 μ g EB/kgBW/day into ovariectomized rats was sufficient to return the plasma estradiol to levels similar to that found in a sham-operated group (Figures 1 and 2). This amount also resulted in the maintenance of uterine weight expressed as a percentage of body weight equal to that of a sham-operated group (Figure 3).

In all cases, the ovariectomized rats without E2 showed complete atrophy of the uteri indicating that the rats were gonadal steroid-depleted. Uterine weights in the sham-treated group and rats supplemented with estradiol (control group) were also within the normal range. Circulating E2 in the ovariectomized group was reduced from a mean value of 57.6 pg/mL in the control animals to 24.1 pg/mL in the ovariectomized rats. In ovariectomized animals fed with 20 mL YCJ /kg BW, the mean level of circulating E2 was 40.67 pg/mL and

Table 2. Effect of YCJ on rat wound healing

Treatment	% Marked improvement	% Moderate improvement 2	% Least improvement 3	Recovery time of wound healing	Inflammation during the wound healing	Scarring
Ovx	0	50	50	+++	+++	1
Sham	40	60	0	++	++	1
Control	0	90	10	+++	+++	1
20 mL	60	40	0	++	++	0
100 mL	90	10	0	+	+	0
100 mL + EB	3 70	30	0	+	+	0

Table 1 The accelerating rate of wound healing on the lateral sides of rats presented as percentage of each group. 1 Marked improvement means the wound had less edema, less reddish soft tissue, and dry. The wound closure also took a shorter time than the other 2 groups, approximately 9-11 days after ovx. 3 Least improvement means the wound had severe edema, very red subcutaneous tissue and wet. Wound closure took a longer time than the other 2 groups approximately 18-21 days after ovx. 2 Moderate improvement had all the above signs shown by the other 2 groups and it took approximately 12-17 days after ovx for the wound to be completely closed. Three symptoms, recovery time, state of inflammation and scarring were scored as +++ = the worst or the longest period of time (18-21 days after ovx operation), + = the best or the shortest period of time for all symptoms (9-11 days after ovx operation). Scarring was evaluated on the day of sacrifice after 6 weeks of ovariectomy: 0 = no scar, 1 = having scar. Control = ovx + E2 2.5 μ g/kg BW/day; 20 mL = ovx+YCJ feeding 20 mL/kg BW/day; 100 mL = ovx+YCJ feeding 100 mL/kg BW/day; 100 m



Figures 6-9. Illustrations of wound healing on the lateral sides of rats. All animals fed with YCJ at 100 mL/kg BW/day had wound healing faster than those non-feeding with YCJ (100%).

Figure 6 and 7 = ovx + YCJ 100 mL/kg BW

Figures A: 1 day after YCJ feeding Figures B: 3 day after YCJ feeding Figures C: 8 day after YCJ feeding Figures D: 11 day after YCJ feeding

[Color figure can be viewed in the electronic version]

in those fed with 100 mL YCJ /kg BW, the mean level was 65.67 pg/mL; the latter value was not significantly different from those of the control groups (sham and ovx plus EB) (p>0.05). Surprisingly, circulating E2 was lowest (22.20 pg/mL) in the ovariectomized rats fed with 100 mL of YCJ plus 2.5 µg/kgBW/day EB injection twice a week, even though their uterine weights were not significantly different from those in the control groups

(Figures 4 and 5). It seems that the presence of exogenous estrogen antagonizes the production of serum E2 from YCJ. Kuiper et al. (1998) and Nikov et al., (2000) have proposed that phytoestrogens and endogenous estrogens compete for the same estrogen receptor sites. In ovariectomized animals fed with 100 mL YCJ/kg BW, the uterine weight was lower than those fed with 20 mL YCJ /kgBW (Figure 5). Both groups fed with YCJ had lower



Figure 8 = $ovx + YCJ 100 \text{ mL/kg BW} + EB 2.5 \mu g/kg BW/day$

Figure $9 = \text{ovx} + \text{EB } 2.5 \,\mu\text{g/kg BW/day}$ This case showed the worst wound of this group.

Figures A: 1 day after YCJ feeding or EB 2.5 µg/kg BW/day subcutaneous injection

Figures B: 3 day after YCJ feeding or EB 2.5 µg/kg BW/day subcutaneous injection

Figures C: 8 day after YCJ feeding or EB 2.5 µg/kg BW/day subcutaneous injection Figures D: 11 day after YCJ feeding or EB 2.5 µg/kg BW/day subcutaneous injection

[Color figure can be viewed in the electronic version]

uterine weights compared with the sham-operated and control groups but the differences were not statistically significant. This study supports cellular mechanisms producing serum E2 from YCJ activated by phytoestrogens which appear to be concentration dependent (Wang and Kurzer, 1997). It is of interest that Rice et al. (1995) found that consumption of high amounts of tofu can block the beneficial effects of estrogen replacement therapy by acting as an estrogen antagonist. There are two classes of phytoestrogens: lignans and isoflavones.

Lignans are present in measurable levels in many fiber-rich foods while isoflavones are confined to legumes, particularly soybeans including soy products and tofu (Whitehead 1996). E2, nevertheless, helped to restore uterine weights in ovariectomized rats fed with 100 mL of YCJ plus 2.5 µg EB injection twice a week. This model would be of interest to minimize the use of exogenous E2 by supplementing with YCJ to preserve uterine weight in oophorectomized patients. Furthermore, the huge doses of the phytoestrogens required is far larger than could be obtained by diet alone. It is also of interest to establish if ovx rats fed with YCJ had a significant decrease in bone loss similar to that obtained with genestein in soybean (Arjmandi et al., 1996) or ipriflavone, a synthetic nonhormonal drug produced commercially from the isoflavone daidzein. Gambacciani (1997) found that with their model system the most beneficial effects in reducing bone loss and vertebral bone density were seen in postmenopausal women who received a low-dose of ipriflavone with estrogens and ipriflavone with calcium. In the present study, ovx rats were fed with YCJ plus EB 2.5 µg twice a week. Even though the bone density was not measured in the present study, it was proved that the supplementation of EB 2.5 µg injection twice a week was enough to restore the uterine weight in ovx fed with YCJ 100mL/kg BW group (Figure 5).

Using a radioimmunoassay technique, Punghmatharith (1988) found that 1 mL of coconut juice contained 2.45 pg of 17β-estradiol and also other sex hormone-like substances, i.e. estrone-3-glucuronide (280.27 pg), pregnanediol-3αglucuronide (263.27 pg), progesterone (27.17 pg), testosterone (1.58 pg) and estrone (0.75 pg). Results from thin-layer chromatography showed that young coconut juice contained substances similar to estrone, 17β -estradiol and β -sitosterol (Punghmatharith, 1988). She also found that subcutaneous injection of an ethereal extract of YCJ reconstituted at a dose equivalent to 7,500 mL of young coconut juice/kg BW/day for 3 consecutive days significantly increased the uterine wet weight of immature rats.

If it was assumed that 1 mL YCJ contains 2.45 pg E2 then in the present study feeding the equivalent of 100 mL/kg BW/day would provide 245 pg E2. From Figure 4, ovx rats fed with 100 mL YCJ showed a significant E2 level compared with ovx group. The extra serum E2 might come from other constituents in YCJ e.g. estrone-3-glucuronide, and estrone. Perhaps it is this process that is inhibited by the injection of EB twice a week, as E2 levels in ovx rats fed with 100 mLYCJ/kg BW/day plus an EB (injection) at 2.5 μ g/kg

BW/day twice a week did not show a significant difference from the non-injected ovx control group.

It would be of interest to use sham-operated animals fed with YCJ to see how YCJ would interact with endogenous estradiol. It would also be extremely useful to have the amounts of E2 present in YCJ confirmed by a sensitive HPLC or other technique in addition to the current CIA method.

In these experiments we noted that ovariectomized rats receiving YCJ at 100 mL/kg BW showed a much better wound healing effect as well as having brighter skin and softer hair than other groups (Table 1 and Figures 6, 7, 8 and 9). This might be due to the estrogen effect of YCJ reported by other research workers. For example Pirila et al. (2001, 2002) found that estrogen can promote wound healing in ovariectomized rats. There is also evidence from animal studies dating back to 1962 that estrogen plays a crucial role in cutaneous wound healing and repair is significantly delayed in its absence (Ashcroft et al., 1997, 1999; Calvin et al., 1998; Jorgenson and Schmidt 1962; Pallin et al., 1975). There have been reports showing that HRT prevents the development of chronic wounds (both pressure ulcers and venous ulcers) in postmenopausal women (Berard et al., 2001; Margolis et al., 2002). Estrogen accelerates the cutaneous wound-healing process, associated with enhanced matrix deposition, rapid epithelialization, and a dampening of the inflammatory response (Ashcroft et al., 1997, 1999; Calvin et al., 1998; Jorgenson and Schmidt 1962; Pallin et al., 1975; Pirila et al., 2001, 2002). At a molecular level, research has also shown that sex hormones have a regulatory effect on growth factors involved in the wound healing process such as the keratinocyte growth factor (Rubin et al., 1995). Furthermore, YCJ contains β-sitosterol (58%) and also other sterols i.e. stigmastatrienol (4.5%), stigmasterol and fucosterol (31.5%), α-spinasterol and others (6%) (Punghmatharith, 1988). β-sitosterol is structurally related to animal cholesterol and can possibly act as a precursor of sex steroids (Moghadasian, 2000). β-sitosterol was found, at physiological levels, to stimulate the release of

prostacyclin (PGI2) from rat vascular smooth muscle cells (Awad et al., 2001). PGI, has been shown to act as a vasodilator, anti-platelet aggregator (Moncada, 1982) and posesses analgesic and anti-inflammatory properties (Bley et al., 2006). A sterol fraction composed of campesterol (7.6%), stigmasterol (28.4%) and β-sitosterol (61.1%) showed anti-inflammatory activity in in vivo murine models of inflammation (Navarro et al., 2001). Many reports also have indicated that βsitosterol and its glucoside stimulate human peripheral blood leukocyte proliferation, and significantly increase the activity of helper T-cells, cytokines, interleukin 2, γ-interferon, and NK cells and thereby are useful in the therapy of several diseases caused by immune dysfunction (Bouic et al., 1997). From this basic knowledge, it is possible that the accelerating rate of wound healing in ovx rats treated with YCJ resulted from the estrogenlike hormones and plant sterols present in YCJ. Histological and cellular mechanism studies, designed to confirm the wound healing effect of YCJ are in progress. Chemical structures of phytoestrogen in YCJ are being analysed.

Conclusions

To the best of our knowledge, this is the first study to investigate the effects of YCJ *in vivo* particulary the effect on wound healing. These findings indicate that estrogen-like hormones in YCJ have an antagonistic effect with endogenous estradiol by competing for estrogen receptors. These findings also indicate that YCJ may find use as an alternative agent for the hormone replacement therapy or postmenopausal symptoms. Further investigations on the effects of YCJ in delaying or halting the progression of Alzheimer's disease are underway. In addition, substances in YCJ were shown to have estrogen-like wound healing activities in rats.

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