Labisia Pumila has Similar Effects to Estrogen on the Reproductive Hormones of Ovariectomised Rats.

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Citation

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Abstract

Introduction: The use of estrogen replacement therapy (ERT) in postmenopausal women has been linked to increased risks of endometrial and breast cancer. Labisia pumila (LP) which has been used traditionally for women's health is a potential alternative agent for ERT. In this study we have compared the effects of LP aqueous extract to ERT (Premarin®) on reproductive hormones using ovariectomized rat model. Methods: Thirty-four ovariectomized rats were divided into five groups, A, B, C, D and E. Group A (the ovariectomized-control group) was given distilled water. Group B was given Premarin 0.07 mg/kg body weight. Groups C, D and E were given LP at doses of 17.5, 35.0 and 70.0 mg/kg body weight, respectively. All treatments were given by daily oral gavages. Blood samples were taken through the tails at 30, 60 and 90 days of treatment for plasma follicle stimulating hormone (FSH), estradiol, luteinizing hormone (LH), testosterone, androsteinedione and dehydroepiandrosterone sulphate (DHEA-S) analysis.Results: The results showed that 60-day treatment with LP at doses of 17.5 mg/kg body weight resembled the effects of Premarin whereby there were significant elevation of estrogen and testosterone levels, suppression of FSH and LH levels compared to ovariectomized-control group. The androsteindione and DHEA-S levels were not altered. Other doses or duration of treatment with LP gave inconsistent results. Conclusions:
LP has shown potential as alternative to ERT which would require further study. The equivalent human dose of LP should be considered based on the dose of 17.5 mg/kg body weight and 60 days of treatment in ovariectomized rats.

INTRODUCTION

Menopause is defined as permanent cessation of the menstrual cycle due to the loss of ovarian follicular activities. It occurs naturally in women between the age of 45 to 52 years (1) or by total ovariectomy (2). The declining ovarian follicular activities in postmenopausal women resulted in decreased estrogen level (3,4,5). This is followed by a high FSH and LH levels due to loss of negative feedback by estrogen (3,6). The testosterone, androsteinedione and dehydroepiandrosterone levels were also lowered in postmenopausal women (7). Postmenopausal women may experience vasomotor symptoms such as hot flushes, night sweats, insomnia, emotional changes, lethargy, vaginal dryness, dyspareunia and urinary incontinence (8). The current treatment for postmenopausal symptoms and diseases related to estrogen deficiency is hormone replacement therapy (HRT) which contains estrogen and progestin. However, HRT has been shown to increase the risks of breast cancer (9, 10, 11) and endometrial cancer (10, 12). The risks of endometrial cancer can be reduced if progesterone is added to the HRT (10, 12) but this may further increase the risk of breast cancer (10, 11) and reduced the vasoprotective effects of HRT (13, 14). Following reports from Women's Health Initiative (WHI) study that showed an increase risk of breast cancer among postmenopausal women who were using HRT, the prescriptions for HRT have declined rapidly. The WHI study reports were supported by s recent findings that the decline in the use of HRT may be related to the decline in the rate of new breast cancer cases (15). The age-adjusted breast cancer incidence rates in women in the United State fell 6.7% in 2003 (16).

In our quest to find alternative treatment to HRT, we have identified Labisia pumila (LP), a member of the small genus of slightly woody plants of the family Myrsinaceae (17) as the candidate to replace HRT. There is only one published report on the estrogenic effects of LP ethanolic extract on Ishikawa-Var I (18). LP has been used for generations as women's health supplement in Malaysia. Phytoestrogen in plants resemble the chemical structure of estrogen and can function like estrogen (19, 20). LP is safe and does not pose

any significant reproductive toxicity or complication during pregnancy, delivery and early pup growth in rats. The No Observable Adverse Effect level (NOAEL) of the extract in is 800 mg/kg/day (21). The LD_{50} (lethal dose 50) of the extract is more than 5 g/kg body weight and the extract produced no significant adverse effects. No deleterious effects were observed in a chronic toxicity study in rats.

The purpose of this study is to compare the effects of LP on reproductive hormones to the standard treatment i.e. estrogen. Ovariectomized rat was used as the postmenopausal model. If LP can emulate the effects of estrogen on the reproductive hormones in the model, then it has potential as alternative to estrogen in the treatment of postmenopausal symptoms and estrogen deficiency-related disease.

MATERIAL AND METHODS

Prior ethical clearance was obtained from the UKM Animal Care and Use Committee (PP/FAR/2009/NAZRUN/14JULY/267-JULY2009-MAY-2010) and the experiment was conducted at Animal Unit, Institute for Medical Research, Malaysia. Thirty female Sprague-Dawley rats, aged 10 to 13 months and weighing between 250 – 350 g were ovariectomized and randomly divided into 5 groups; ovariectomized-control (OVXC), estrogen replacement at 0.07 mg/kg body weight Premarin® (ERT), LP treated groups at doses of 17.5 mg/kg body weight (LP17.5), 35.0 mg/kg body weight (LP35) and 70.0 mg/kg body weight (LP70). OVXC group was only given distilled water as a vehicle. Labisia pumila var alata was extracted in the Forest Research Institute of Malaysia (FRIM) in powder form. All treatments were given daily by oral gavages at 9.00 a.m. for 90 days.

Premarin® (Wyeth, Co. Kildare, Ireland) was crushed and dissolved in distilled water. Every 0.5 ml of gavage contained 0.07 mg/kg body weight of Premarin®. This dose was calculated based on the human dosage (22). Labisia pumila powder was also dissolved is distilled water. Every 0.5 ml of gavage will give the doses of 17.5, 35.0 or 70.0 mg/kg body weight of LP, depending on the treatment groups. Blood samples were collected into EDTA tubes from the tails before treatment and at 30, 60 and 90 days of treatments. It was left for 30 minutes and centrifuged at 3000 r.p.m. for 5 minutes to separate the plasma. The plasma was stored at -20 °C. Plasma follicle stimulating hormone (FSH) was measured using immunoradiometric assay (IRMA) while plasma estradiol, luteinizing hormone (LH), testosterone, androsteinedione and dehydroepiandrosterone sulphate (DHEA-S) were measured using radioimmunoassay (RIA). The kits used were Ultra-sensitive Estradiol RIA (DSL-4800), rat FSH IRMA (AH R004), rat LH RIA (AH2002), testosterone RIA (DSL-4100), androstenedione RIA (DSL-3800) and DHEA-S RIA (DSL-3500).

STATISTICAL ANALYSIS

Data analysis was performed using Statistical Package for Social Sciences software (version 16.0; SPSS Inc., Chicago). Data were tested for normality using Kolmgorov-Smirnov test. If the data was normally distributed, analysis of variance (ANOVA) with Tukey's test was performed. For non-normally distributed data, non-parametric Kruskall-Wallis and Mann-Whitney U tests were performed and data were expressed as median and range.

RESULTS

The median estradiol levels for OVXC group at 30, 60 and 90 days of treatment were 26.59 (23.16-32.26), 18.21 (17.62 – 21.34) and 20.51 (17.05 – 24.44) pg/ml, respectively. As expected ERT group had consistently higher estradiol levels of 47.64 (40.04 – 95.59), 66.59 (34.32 – 111.32) and 24.90 (24.57 – 27.44) pg/ml, at the corresponding treatment period compared to OVXC group ($p<0.05$). There were no significant differences in the estradiol levels for all three LP-treated groups at 30 days of treatment. However, after 60 days of treatment, only the median estradiol of LP17.5 group (35.51 (19.45 – 69.73) pg/ml) was significantly higher than OVXC group ($p<0.05$) and comparable to ERT group. Yet, after 90 days of treatment, Labisia pumila at all doses failed to raise the estradiol level (Table 1).

As for the FSH level, ERT group had lower FSH level of 53.37 (51.32 – 55.04) and 46.77 (32.67 – 62.14) ng/ml at 60 and 90 days of treatment respectively compared to ERT group ($p<0.05$). Surprisingly, all LP-treated groups had lower FSH levels compared to OVXC group ($p<0.05$) at 30-day treatment. In fact, LP35 group had significant lower FSH level than ERT group ($p<0.05$) during this time. The low FSH levels were maintained at 60 and 90 days of treatments except to LP70 group, which failed to do so at 60 days of treatment (Table 2).

LH levels for all the groups were similar at 30 days of treatment. ERT Group had a significantly lower plasma LH level compared to OVXC group ($p<0.05$) only at 90 days of treatment, whereas LP17.5 group showed significantly lower LH level at 60 days of treatment compared to OVXC group

(p<0.05) and ERT group (p<0.05). However, all the LP-treated groups failed to lower LH level at 90 days of treatment (Table 3).

Testosterone level for ERT group was raised at 60 days of treatment compared to OVXC group (p<0.05). Surprisingly, LP35group was the earliest group to raise testosterone level at 30 days of treatment compared to OVXC group, but failed to maintain the level after that. Similar to ERT group, testosterone levels in LP17.5 group was raised at 60 days of treatment compared to OVXC group (p<0.05). All the groups have similar testosterone level at 90 days of treatment (Table 4).

Androsteinedione and DHEA-S levels for all groups at all duration of treatments were similar. On the other hand, both LP35 and LP70 groups showed lower level of DHEA-S compared to OVXC group (p<0.05) at 60 days of treatment (Table 4).

Figure 1

Table 1 shows plasma estradiol levels (ng/ml) in ovariectomized rats treated with (). Data are shown as median (range).

Treatment Groups	Duration of treatment		
	30-day	60-day	90-day
A	25.16	18.21	20.51
(Control)	(23.16 - 32.26)	(17.62 - 21.34)	(17.05 - 24.44)
B	47.64 *	66.59 *	24.90 *
(Premarin)	(40.04 - 95.59)	(34.32 - 111.32)	(24.57 - 29.44)
C	23.28	35.51 *	28.82
LP (17.5 mg/kg)	(20.59 - 47.43)	(19.45 - 69.73)	(18.28 - 45.71)
D	28.45	19.34	22.36
LP (35.0 mg/kg)	(20.95 - 52.47)	(17.34 - 27.08)	(17.32 - 33.80)
E	17.53	20.53	34.86
LP (70.0 mg/kg)	(15.59 - 30.00)	(15.76 - 25.57)	(19.42 - 126.07)
* p<0.05 compared to group A			

Figure 2

Table 2 shows plasma follicle-stimulating hormone (FSH) levels in ovariectomized rats treated with (). Data are shown as median (range) of FSH levels in ng/ml.

Treatment Groups	Duration of treatment		
	30-day	60-day	90-day
A	66.42	69.22	88.82
(Control)	(59.14 - 85.73)	(63.50 - 72.76)	(74.92 - 101.35)
B	53.8	53.37 *	46.77 *
(Premarin)	(40.76 - 62.23)	(51.32 - 55.04)	(32.67 - 62.14)
C	45.72 *	50.18 *	65.09 *
LP (17.5 mg/kg)	(15.32 - 56.30)	(10.18 - 63.80)	(38.24 - 74.37)
D	37.47 #	52.3	60.37 *
LP (35.0 mg/kg)	(5.97 - 48.74)	(36.67 - 69.13)	(36.07 - 77.14)
E	48.08 *	54.68	62.17 *
LP (70.0 mg/kg)	(40.81 - 54.09)	(32.09 - 85.86)	(41.04 - 90.04)
* p<0.05 compared to group A			
# p<0.05 compared to group A and B			

Figure 3

Table 3 shows plasma luteinizing hormone (LH) level in ovariectomized rats treated with (). Data are shown as median (range) of LH levels in ng/ml.

Treatment Groups	Duration of treatment		
	30-day	60-day	90-day
A	4.30	3.31	4.38
(Control)	(4.14 - 4.35)	(3.01 - 3.90)	(2.59 - 6.95)
B	3.12	3.43	2.14 *
(Premarin)	(2.08 - 5.38)	(2.65 - 4.91)	(0.15 - 3.71)
C	2.9	1.06 #	2.66
LP (17.5 mg/kg)	(2.83 - 3.08)	(0.30 - 2.97)	(2.21 - 3.17)
D	3.1	4.03	4.21
LP (35.0 mg/kg)	(0 - 5.3)	(1.57 - 5.95)	(2.87 - 5.24)
E	4.14	4.25	4.75
LP (70.0 mg/kg)	(4.10 - 4.49)	(3.17 - 4.60)	(3.46 - 5.43)
* p<0.05 compared to group A			
# p<0.05 compared to group A and B			

Figure 4

Table 4 shows plasma androgen levels in ovariectomized rats treated with (). Data are shown as median (range).

Treatment Groups	Testosterone level (ng/ml)			Androstenedion level (ng/ml)			DHEA-S level (µg/ml)		
	30-day	60-day	90-day	30-day	60-day	90-day	30-day	60-day	90-day
A (Control)	0.00 (0.001-0.002)	0.01 (0.001-0.009)	0.008 (0.001-0.026)	0.12 (0.02-0.20)	0.12 (0.02-0.12)	0.16 (0.11-0.17)	0.13 (0-1.3)	0.70 (0.3-22.5)	1 (0.9-1.4)
B (Premarin)	0.008 * (0.001-0.021)	0.040 * (0.011-0.096)	0.027 * (0.022-0.051)	0.1 (0.02-0.17)	0.23 (0.04-0.34)	0.2 (0.05-0.33)	0.9 (0-3.1)	3.3 (1.5-7.2)	1.5 (0.7-17.4)
C *LP* (17.5 mg/kg)	0.004 (0.001-0.010)	0.043 * (0.041-0.057)	0.03 (0.014-0.169)	0.12 (0.03-0.49)	0.12 (0.07-0.23)	0.19 (0.04-0.37)	0.9 (0.5-1.5)	1.2 (0.6-9.7)	1 (0.8-12.4)
D *LP* (35.0 mg/kg)	0.016 * (0.001-0.216)	0.005 (0.001-0.033)	0.024 (0.007-0.081)	0.19 (0.14-0.21)	0.1 (0.03-0.24)	0.24 (0.02-0.41)	1.2 (0.4-4.5)	1.4 * (0-3.8)	1 (0-3.4)
E *LP* (70.0 mg/kg)	0.009 (0.001-0.028)	0.002 (0.001-0.008)	0.036 (0.001-0.082)	0.08 (0.01-0.20)	0.13 (0.02-0.17)	0.13 (0.02-0.28)	0.8 (0.6-3.5)	1* (0.3-8.8)	1.6 (1.0-2.7)
* p<0.05 compared to group A									
*p<0.05 compared to group B									

DISCUSSION

In response to the WHI report, many physicians have recommended postmenopausal women to stop taking ERT. Prescription of Premarin® and Prempro®, the two most commonly prescribed HRT in the United States, had declined from 61 millions prescriptions in 2001 to 21 millions in 2004 (16). As the popularity of HRT continues to drop, postmenopausal women increasingly choose alternative forms (23).

Based on the effects of LP on the reproductive hormones of ovariectomized rats, an accepted postmenopausal model, this natural product may have potential as alternative to HRT. To qualify as one, it should be able to mimic the effects of estrogen on the reproductive hormones. Of the three different doses of LP and the three different duration of treatment, LP at the dose of 17.5 mg/kg body weight given for 60 days had exhibited the closest resemblance to estrogen replacement.

It was also noticed from the results that the ovariectomized control group has minor variation in the estradiol levels.

When Premarin® or LP was given, there was an increasing variability in the esradiol levels within the group. This indicated that the response of the individual rat to the treatments may vary, giving rise to the variations in the estradiol levels.

Of all the reproductive hormones, the most important hormone which needs attention is estradiol as this hormone is deficient in postmenopausal women and needs to be replaced. Premarin® has resulted in an increase in estradiol level as early as 30 days of treatment compared to 17.5 mg/kg body weight of LP which requires 60 days of treatment before it raised the estradiol level. This is expected for Premarin® as its active ingredient is conjugated estrogen which can directly replace estradiol in ovariectomized rats. The delayed rise in estradiol level suggested that LP must have another mechanism which required some time to affect the production of estradiol. This mechanism has yet to be discovered. During menopause, adrenal glands replace ovaries as the main source of estrogen production in the form of estrone which may also be aromatized to a more potent estradiol (3). The other sites of estrone production are the liver and adipose tissues. Labisia pumila may have stimulated these tissues especially the adrenal glands to produce more estrone or it could also stimulate the aromatization of estrone to estradiol.

Labisia pumila treatment was also found to be effective in lowering FSH levels. Almost all doses and duration of treatment with LP has managed to lower the FSH levels, similar to the treatment with Premarin. The suppression of FSH levels is thought to be due to the raised estrogen level by Premarin or LP which produced negative feedback to the anterior pituitary gland. Therefore, the lowered FSH level should be preceded by a raised estradiol level. What is puzzling is that, the FSH was lowered earlier than the rise of estradiol in LP17.5 group. In fact, the other LP35 and LP70 groups have managed to lower the FSH without exhibiting any effects on the estradiol levels. This finding further complicates the understanding on the mechanism of action of LP. Elevation of FSH in postmenopausal women is believed to be responsible for the menopausal symptoms such as hot flushes (24). Therefore, the reduction in FSH by LP may be useful in treating these menopausal symptoms as has been shown by other phytoestrogens, which were effective in reducing hot flushes (19, 25, 26).

The negative feedback of the estrogenic effects on the hypothalamus and pituitary gland should have resulted in similar pattern for the other gonadotropins, LH. However, only LP17.5 group and ERT group had low LH levels at 60 and 90 days of treatment, respectively. The different pattern compared to FSH may be caused by the shorter half life and the faster excretion of LH from the body (27, 28, 29).

Testosterone is believed to play an important role in women's libido (30,31). The drop in testosterone in postmenopausal women may be responsible for the loss of libido. In fact, women who had undergone ovariectomy showed significant loss of libido compared to natural occurring postmenopausal women as they have a more significant drop in testosterone level (32, 33). Similar to estrogen replacement therapy, LP may restore libido in postmenopausal women by raising the testosterone level, as both LP and Premarin had no effects on androsteinedione and DHEA-S levels in the ovariectomized rats.

LP supplementation had resembled the effects of estrogen replacement therapy on reproductive hormones in our postmenopausal rat model. The LP dose of 17.5 mg/kg and 60 days of treatment had successfully raised the estradiol and testosterone levels and lowered the FSH and LH levels. Therefore, this LP dose is suitable for treatment of menopausal symptoms and estrogen deficiency-related diseases. While, the LP dose of 35 mg/kg was able to increase the testosterone level and reduce FSH level at 30 days of treatment. This dose would be useful to boost the libido and reduce the menopausal symptoms. Considering these facts, we propose two regimens of LP, starting LP at the dose of 35 mg/kg body weight for 30 days to benefit its fast effects on libido and menopausal symptoms, followed by reduction of the LP dose to 17.5 mg/kg body weight to maintain its therapeutic effects especially the raised estradiol levels. The safety of LP is another important issue that has to be considered. The acute, subacute and chronic toxicity studies found that LP is safe while a reproductive toxicity study found no gross visceral changes of the ovaries or uterus (34, 35, 36). However, a carcinogenic study is still required to exclude its possibility of causing cancer.

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