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Safety and efficacy of a *Labisia pumila* var *alata* water extract on sexual well being and lipid profile of pre- and postmenopausal women: A randomized double-blind pilot study

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This randomized double-blind, placebo-controlled study investigated the safety and efficacy of *Labisia pumila* (*LP*) water extract on sexual health, lipid profile and inflammatory markers in 36 healthy pre-and post-menopausal North American women. Participants were randomized to either *LP* (200 mg) or placebo for 12 weeks. The female sexual function index (FSFI) and short form-36 health survey (SF-36) were completed, and lipid profiles, anti-inflammatory markers, urinary antioxidants and safety parameters were assessed. There were no significant differences in FSFI and SF-36 scores after 12 weeks. Compared to placebo, women on *Labisia pumila* trended towards a reduction in total cholesterol after 12 weeks (p=0.077). Urinary 8-isoprostane concentrations from baseline to week 12 decreased for both groups, with women on *L. pumila* demonstrating a greater decrease (Δ= -144.4nmol/L) versus placebo (Δ= -125.9nmol/L). Significant decreases in serum IL-6 from baseline to week 6 were observed in *Labisia pumila* and placebo (p=0.006 and p=0.012 respectively) but these differences were not sustained through week 12. *LP* demonstrated a trend towards an improvement in TC, urinary 8-isoprostane and significant within group improvement in IL-6 and IL-1β suggesting a role for *LP* in improving inflammation. Future research should focus on older subjects that are sexually dysfunctional.

Key words: *Labisia pumila*, women's health, randomized double-blind trial, female sexual function index, blood lipid profile, cytokines.

INTRODUCTION

A high proportion of North American women experience low sexual desire, difficulties with orgasm and painful and un-pleasurable intercourse (Pujols et al., 2010). The National Health and Social Life Survey, a well-designed, large population-based study on adults ranging from ages 18 to 59, found a high overall prevalence of female

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Abbreviations: LP, *Labisia pumila*; **FSFI,** female sexual function index; **AEs,** adverse events; **ALT,** alanine transaminase; **AST,** aspartate aminotransferase; **BMI,** body mass index; **CBC,** complete blood count; **GGT,** gamma-glutamyl transferase; **HDL-C,** high density lipoprotein cholesterol; **ICH,** International Conference of Harmonization; **IL,** interleukin; **LDL-C,** low density lipoprotein cholesterol; **SF-36,** Short Form 36; **SOD,** superoxide dismutase; **TNFα,** tumor necrosis factor-alpha.

sexual dysfunction in US women (Goldstein et al., 2004). Sexual dissatisfaction was associated with sexual dysfunction, and women who reported greater distress over sexual problems also reported greater sexual dissatisfaction (King et al., 2007).

Though satisfaction with sexual function in women has not been well-described, and little is known of the differences in sexual function between pre- and post-menopausal women (Davison et al., 2008), sexual activity has been shown to decline with age (Dennerstein et al., 2005). In studies with methodology that allows the effects of menopausal status to be separated from aging, it is clear that there is a further effect on sexual function of menopausal status over that of aging (Dennerstein et al., 2005). Estrogen secretion declines to very low levels post-menopause; this results in vulvovaginal atrophy and can lead to sexual pain and trauma during intercourse (Buster, 2013). Neuroendocrinal effects of declining estrogen levels, including mood swings, hot flushes, irritability, memory lapses and insomnia also adversely affect sexual response (Buster, 2013). As well, there are some evidence to suggest a link between sexual dysfunction and cardiovascular disease (Archer et al., 2005; Steinke, 2010).

Labisia pumila var *alata* (family Myrsinaceae), is a well-known and popular herb for feminine vitality, sexual wellbeing and hormonal balance in South East Asia (Burkill et al., 1966; Gimlett, 1971; Lemmens et al., 2002). Previous *in vitro* and *in vivo* studies report that extracts of *L. pumila* (*LP*) have various immunomodulatory (Pandey et al., 2008), antioxidant (Tasduq et al., 2007), lypolytic (Al-Wahabi et al., 2007) and aphrodisiac effects (Asiah et al., 2007). Several bioactive constituents have been identified in the extract including benzoquinones (Houghton et al., 1999), alkenyl resorcinols (Jamal and Houghton, 1999) and triterpenoids (Jamal, 2006), as well as flavonoids (apigenin, kaempferol, rutin and myricetin), isoflavonoids and phenolic compounds (gallic acid, pyrogallol and caffeic acid) (Chua et al., 2011; Karimi and Jaafar, 2011; Karimi et al., 2011; Chua et al., 2012). Water extracts of *LP* have long been used for gynecological issues in traditional medicine (Ismail et al., 1999), but there is limited clinical evidence on safety and efficacy. Traditionally, this herb is taken for pre-menstrual problems, post-labor tonic and a general tonic for women's health presenting uses in women of varying ages (Burkill et al., 1966; Gimlett, 1971; Ismail et al., 1999). Malay women use *LP* for muscle pain, uterine health and sexual satisfaction (Intan et al., 2005). A previous study on postmenopausal women showed that *LP* extract was safe (Nik Hazlina et al., 2009a, b).

The aim of this 12-week pilot study was to provide further evidence on the efficacy of *LP* on sexual well-being, quality of life and cardiovascular health and safety in North American women. A wide age group, from 18 to 70 years of age, was considered for this study to determine the group likely to benefit from *LP* supplementation.

MATERIALS AND METHODS

Ethical approval of the study

This study was conducted in accordance with the Guideline for Good Clinical Practice (ICH-6) and Declaration of Helsinki. Authorization was received from the Natural Health Products Directorate on December 09, 2009 and unconditional approval was granted by Institutional Review Board Services, Aurora, Ontario, Canada on January 08, 2010. The study was conducted at a single site; KGK Synergize Inc., London, Ontario, Canada.

Study population, sampling and recruitment

The pilot study was a single-site, randomized, double-blind, placebo controlled 12-week parallel study in 36 healthy women. Women were recruited from a research subject database and advertisements in newspapers.

Women were included in the study if they were between 18 and 70 years of age, regardless of level of sexual activity, were healthy as determined by laboratory results, medical history and physical examination and gave informed written consent. Women were excluded if they reported sexual dysfunction, were on hormone therapy, allergic to study products, pregnant or breast feeding, or had a history of breast, uterine or ovarian cancer, autoimmune conditions, immunodeficiency, history of bleeding disorders, gynecological disease, any serious gastrointestinal, hepatic, renal, cardiovascular, neurological or hematological disorder; drug or alcohol abuse or on natural health products/dietary supplements within two weeks prior to randomization.

Randomization and intervention

Subjects were randomly assigned to two treatment groups in a 1:1 ratio using computer-generated randomization tables into 18 blocks of two to receive either one tablet of *LP* water extract or a matching placebo, once daily for 12 weeks. Randomization was stratified based on menopausal status; pre-menopausal or post-menopausal (>1 year since last menstruation).

Pre-menopausal women were randomized starting from the top of the randomization schedule and postmenopausal women were randomized starting from the bottom of the randomization schedule to prevent an imbalance between groups with respect to menopausal status.

Experimental design and investigational product

After enrollment at baseline (day 0), follow-up visits occurred at six weeks (day 42 ± 3) and 12 weeks (day 84 ± 3). During the intervention, each participant consumed 1 tablet containing either *LP* or placebo in the morning after breakfast. The study product *LP* consisted of 200 mg of aqueous extract of *LP* root and leaves (BIO *LP*101) with polygonum minus, calcium phosphate monobasic, microcrystalline cellulose, hydroxymethyl cellulose, cellulose, silicon dioxide, magnesium stearate, talcum, hypromellose, sodium carboxymethyl cellulose, iron oxide red, titanium dioxide, beet root powder, macrogol 6000, maltodextrin as excipients, and was manufactured under continuous quality control of Good Manufacturing Process requirements (batch number: 30 PED 060; DER: 10:1; Biotropics Malaysia Berhad, Kuala Lumpur, Malaysia). The placebo tablet corresponded to the active medication without the herbal extract. The dosage used in this study was based on both a combination of scientific data from animal studies, using 1/7 species conversion factor, and traditional use studies (Freireich et

al., 1966; Samy et. al., 2005; Kadir et al., 2012).

Primary outcome measure

The primary endpoints were determined by measuring the improvement in quality of life at each visit, using the female sexual function index (FSFI) and RAND Short Form-36 Health Survey (SF-36) questionnaires. The FSFI questionnaire included 19 questions which overlapped six domains: desire, arousal, lubrication, orgasm, satisfaction and pain (Rosen et al., 2000). The SF-36 scale included questions classified in the following domains: Total physical, total mental, physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotion and mental health.

Secondary outcome measure

The secondary outcomes included plasma lipid profile (total cholesterol (TC), high density lipoprotein-C (HDL-C), low density lipoprotein-C (LDL-C), triglycerides), antioxidants (8-isoprostane, serum superoxide dismutase (SOD), anti-inflammatory markers (cytokines TNFα, interleukin (IL)-6, IL-1β), hormones (estradiol-17β), blood chemistry (electrolytes, glucose, creatinine and bilirubin), liver function markers (aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT)), hematology (complete blood count- (CBC), weight and vital signs. The secondary objectives were assessed at baseline and at 6 and 12 weeks of treatment. Two, first-morning void urine samples were collected the day before and the day of the study visit, and were pooled and analyzed for creatinine and 8-isoprostane. Adverse events (AEs) were documented at each visit and were classified according to the description, duration, severity, frequency and outcome. Their relationship to the investigational product was assessed by the Medical Director.

Laboratory analyses

Blood was collected into 4 ml ethylenediaminetetraacetic acid (EDTA) tubes. Serum generated was analyzed to determine hematology, plasma lipid profiles, blood chemistry and liver function endpoints (LifeLabs Medical Laboratory Services, London, ON, Canada). Serum was frozen and stored at -40°C for the measurement of cytokines and SOD by enzyme linked immuno-sorbent assay (ELISA): TNFα (catalog no. 555212, BD Bioscience, Mississauga, ON, Canada), IL-6 (catalog no. 555220, BD Bioscience, Mississauga, ON, Canada), and IL-1β (catalog number 557953, BD Bioscience, Mississauga, ON, Canada) SOD (catalog no. 706002, Caymen Chemical, Ann Arbor, MI, USA). Aliquots of 1 ml of urine were frozen at -40°C for urinary 8-isoprostane analysis by ELISA (catalog no. 51635, Caymen Chemical, Ann Arbor, MI, USA).

Data analysis

No formal sample size calculation was conducted for this study due to the lack of efficacy data for *LP* treatment for quality of life parameters. The planned sample size for this study was 36 participants with 18 women randomized equally to each of the two study arms. Allowing for an anticipated drop-out-rate of 15%, 30 women were expected to complete the trial. Drop-outs during the treatment period or participants leaving the study prematurely were not replaced.

All subjects were included for analysis of safety and efficacy. Between-group comparisons were made using analysis of covariance (ANCOVA) with adjustment for the baseline value. Within-group changes were made using *t*-tests. Fisher's exact test was used for comparing frequencies between groups for categorical data. Compliance, defined as the number of pills taken, in the two groups was analysed using t-test. Probability values ≤0.05 were considered statistically significant.

A subgroup analysis on sexually active women stratified by age (18-29 years, 30-39 years, 40-49 years and ≥50 years) was conducted. During statistical analysis, subjects were classified based on FSFI scores and those with scores ≤26 were considered sexually dysfunctional (Wiegel et al., 2005). An interaction test was conducted to test whether or not the difference between the two groups in one age category was different from that seen in another age category (Pocock and Enderlein, 1985). SAS Version 9.1 was used to perform the statistical analysis.

RESULTS

Participant characteristics

Subject disposition is shown in Figure 1. Baseline demographic and blood chemistry measures were comparable between the two groups at screening, but participants receiving *LP* had significantly lower body weight (p=0.019) and body mass index (BMI) (p=0.04) (Table 1).

At screening, subjects on *LP* also had lower mean fasting glucose levels (p=0.026) than those receiving placebo though the values remained well within normal clinical range, and there was no significant difference in fasting glucose levels between groups at baseline, week 6 and week 12. Three subjects in the *LP* group and four in the placebo group were post-menopausal. Fewer participants in the *LP* group consumed alcohol on a weekly basis versus placebo, which trended towards significance. Mean compliance was 93% in *LP* group and 91% in placebo group.

Female sexual function index (FSFI)

There were no significant differences between groups in the total FSFI score (Table 2) or domain scores for desire, arousal, lubrication, orgasm, satisfaction and pain at baseline or at 6 and 12 weeks of treatment. Within groups, after six weeks of supplementation, subjects on placebo reported significantly higher lubrication (p=0.022), orgasm (p=0.048) and total FSFI scores (p=0.05).

These subjects also showed trends in arousal (p=0.067) and pain (p=0.10) scores at week 6. However significance and trends were not maintained through week 12. At week 12, a greater increase in the mean total FSFI score from baseline was reported by women on *LP* versus placebo (0.8 versus 0.4) (Figure 2).

Short form (36) health survey

There were no statistically significant differences between

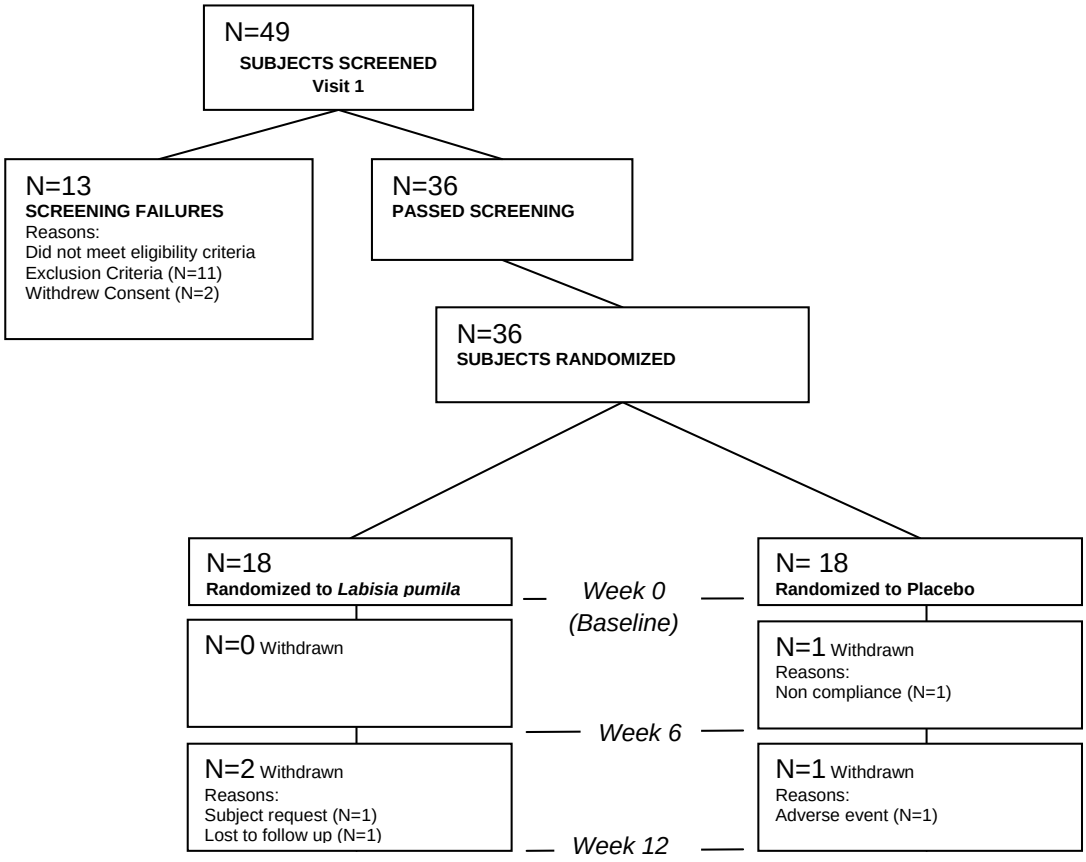

Figure 1. Flow chart showing disposition of study subjects.

Table 1. Demographics and characteristics all subjects randomized into the study.

Parameter	*Labisia pumila* (N=18)	Placebo (N=18)	p-value
	Mean ± SD	Mean ± SD	
Age (years)	39.1 ± 13.0	40.4 ± 12.5	0.756[1]
Weight (kg)	61.2± 7.1	68.6 ± 10.7	0.019[1]
BMI (kg/m^2)	22.8 ± 3.4	25.5 ± 4.3	0.041[1]
Alcohol Use [f/n (%)]			
None	6/18 (33.3%)	1/18 (5.6%)	
Occasional	9/18 (50.0%)	9/18 (50.0%)	0.059[2]
Weekly	3/18 (16.7%)	8/18 (44.4%)	
Tobacco Use [f/n (%)]			
Current	2/18 (11.1%)	0/18 (0.0%)	
Former	2/18 (11.1%)	5/18 (27.8%)	0.241[2]
Never	14/18 (77.8%)	13/18 (72.2%)	

[1]Between group comparisons were made using analysis of variance (ANOVA). Probability values P<0.05 are statistically significant; [2]between group comparisons were made using a *t*-test. Probability values P<0.05 are statistically significant.

treatment and placebo groups with respect to the total SF-36 score or the domains (total physical, total mental, physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotion and mental health) at baseline or after 6 and 12 weeks of treatment (Table 3).

Table 2. Total FSFI score of all subjects randomized into the study at baseline, 6 weeks and 12 weeks.

Parameter	*Labisia pumila*	Placebo	p-value
	[N] Mean ± SD	[N] Mean ± SD	
FSFI Total (score range 2.0 - 36.0, higher is better)			
Week 0 (Baseline)	[17] 24.6 ± 9.7	[15] 26.9 ± 7.7	0.473[1]
Week 6	[17] 25.9 ± 7.5	[17] 28.9 ± 6.1	0.320[2]
Week 12	[15] 24.8 ± 10.2	[16] 26.1 ± 11.3	0.863[2]

[1]Between group comparisons were made using a *t*-test. Probability values P<0.05 are statistically significant; [2]Between group comparisons were made using analysis of covariance (ANCOVA) using baseline as a covariate. Probability values P<0.05 are statistically significant.

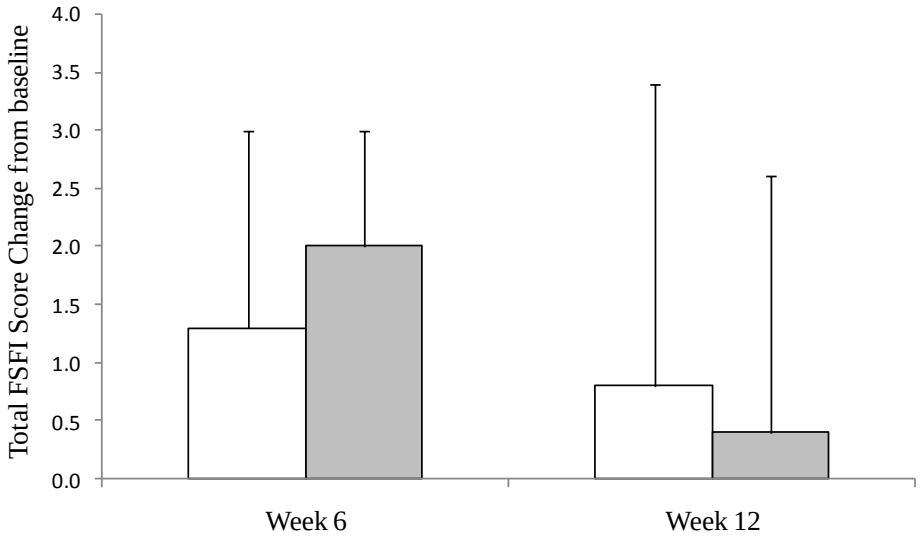

Figure 2. The within-group change of total FSFI score from baseline to week 6 and week 12 for subjects on *Labisia pumila* (white) or placebo (grey).

Table 3. Total SF-36 score of all subjects randomized into the study at baseline, 6 weeks and 12 weeks.

Parameter	*Labisia pumila*	Placebo	p-value
	[N] Mean ± SD	[N] Mean ± SD	
SF 36 Total (Score Range 0.0 - 100.0, Higher is better)			
Week 0 (Baseline)	[18] 89.5 ± 5.9	[18] 90.6 ± 4.9	0.520[1]
Week 6	[18] 88.2 ± 5.2	[18] 87.1 ± 9.8	0.442[2]
Week 12	[16] 88.9 ± 6.1	[17] 88.2 ± 8.7	0.676[2]

[1]Between group comparisons were made using a *t*-test. Probability values P<0.05 are statistically significant; [2]between group comparisons were made using analysis of covariance (ANCOVA) using baseline as a covariate. Probability values P<0.05 are statistically significant.

Lipids

For women on *LP*, TC decreased from baseline to week 12 and when compared to placebo, this trended towards a significant difference (Table 4). Compared to placebo, women on *L. pumila* trended towards a reduction in total

Table 4. Total cholesterol values at baseline, week 6 and week 12 for all randomized subjects.

Parameter	*Labisia pumila*	Placebo	p-value
	[N] Mean ± SD	[N] Mean ± SD	
Total cholesterol (mmol/L)			
Week 0 (Baseline)	[18] 5.0 ± 1.0	[18] 4.9 ± 1.1	0.882[1]
Week 6	[18] 4.9 ± 1.1	[18] 4.9 ± 1.0	0.791[2]
Week 12	[16] 4.8 ± 1.0	[17] 5.2 ± 0.9	0.077[2]
LDL-C (mmol/L)			
Week 0 (Baseline)	[18] 3.0 ± 0.9	[18] 2.8 ± 1.0	0.645 [1]
Week 6	[18] 2.9 ± 0.9	[18] 2.8 ± 0.9	0.648 [2]
Week 12	[16] 2.9 ± 0.8	[17] 3.0 ± 0.8	0.194 [2]
HDL-C (mmol/L)			
Week 0 (Baseline)	[18] 1.5 ± 0.3	[18] 1.7 ± 0.3	0.270 [1]
Week 6	[18] 1.6 ± 0.3	[18] 1.7 ± 0.3	0.197 [2]
Week 12	[16] 1.5 ± 0.3	[17] 1.8 ± 0.3	0.011 [2]
Triglyceride (mmol/L)			
Week 0 (Baseline)	[18] 0.9 ± 0.3	[18] 0.9 ± 0.3	0.538 [1]
Week 6	[18] 1.1 ± 0.6	[18] 0.9 ± 0.3	0.159 [2]
Week 12	[16] 1.0 ± 0.5	[17] 0.9 ± 0.4	0.441 [2]
Total cholesterol/HDL-C ratio			
Week 0 (Baseline)	[18] 3.3 ± 0.8	[18] 3.0 ± 0.7	0.276 [1]
Week 6	[18] 3.2 ± 0.9	[18] 2.9 ± 0.6	0.326 [2]
Week 12	[16] 3.4 ± 0.8	[17] 3.0 ± 0.7	0.435 [2]

[1]Between group comparisons were made using a *t*-test. Probability values P<0.05 are statistically significant; [2]between group comparisons were made using analysis of covariance (ANCOVA) using baseline as a covariate. Probability values P<0.05 are statistically significant.

TCC after 12 weeks (p=0.077) (Table 4). HDL-C increased significantly after 12 weeks of treatment in women on placebo, whereas those taking *LP* maintained baseline levels. The TC/HDL-C ratio was not significantly affected in either group (Table 4).

Estradiol-17β

Changes in serum estradiol-17β were not statistically significant between- or within-groups at baseline, week 6 or week 12 for either treatment. Women aged ≥ 50 years showed the lowest estradiol levels during the study period indicating age-related physiological changes associated with menopausal status (< 130 pmol/L). This age category also demonstrated no change from baseline in hormone levels in either treatment group suggesting that *LP* did not adversely affect estradiol levels.

Oxidative stress markers and cytokines

There were no significant differences in the oxidative stress markers 8-isoprostane and SOD at baseline, week 6 or week 12. However, both groups demonstrated a decreasing trend in urinary 8-isoprostane concentrations from baseline to week 12, with women on *LP* having a greater decrease (Δ= -144.4 nmol/L) versus placebo (Δ= -125.9 nmol/L). Compared to baseline, TNF-α decreased in subjects on placebo and increased in subjects on *LP* after 12 weeks of supplementation; however these changes were not significant. There were significant within-group decreases in serum IL-6 from baseline to week 6 in both *LP* (p=0.006) and placebo (p=0.012) but these differences were not sustained through week 12. IL-1β was significantly decreased in women on *LP* from baseline to week 6 and week 12 (p<0.001, p=0.001) (Figure 3). There were no significant differences between groups in serum IL-6 and IL-1β at baseline, week 6 or week 12.

Subgroup analysis

Subgroup analysis of sexually dysfunctional women was classified by baseline FSFI scores ≤26, but sexually active for the duration of the study, showed that women taking *LP* had significantly higher “orgasm” scores versus

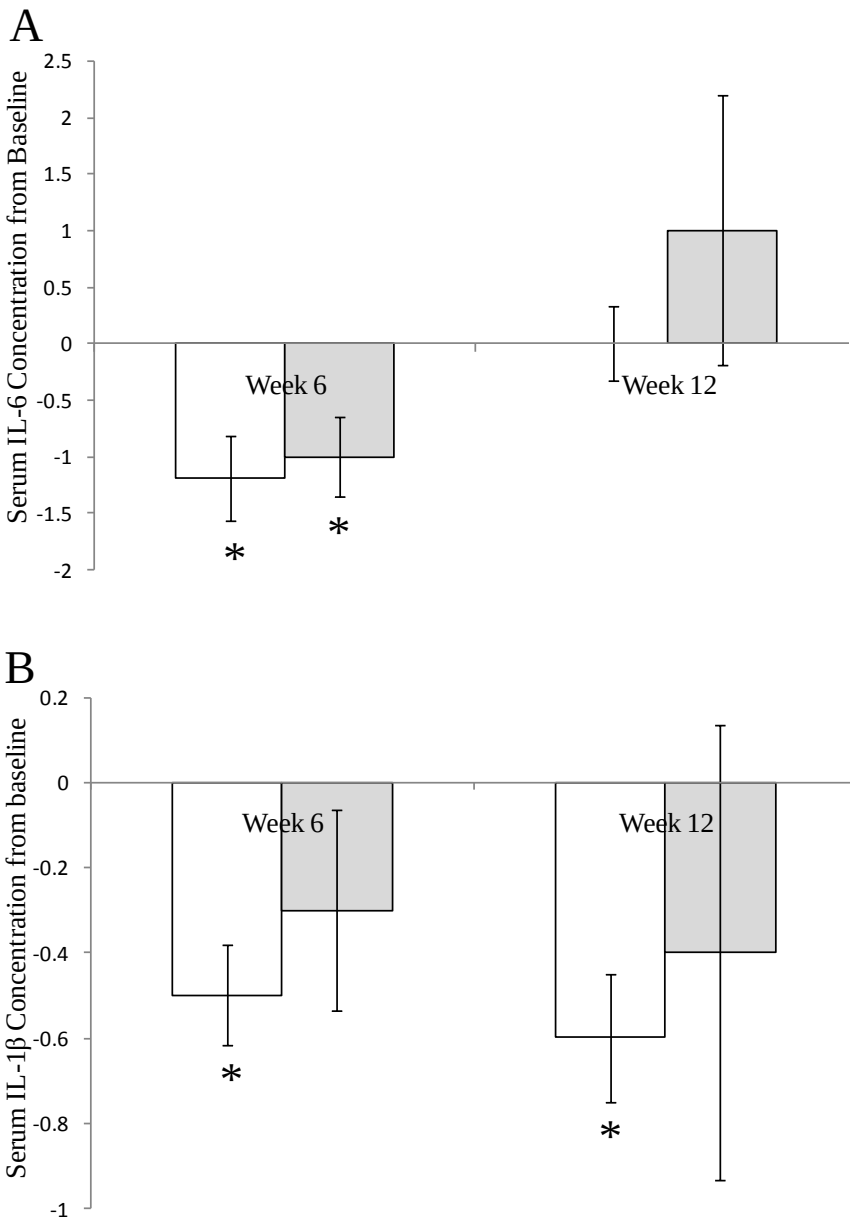

Figure 3. The change from baseline of serum (A) IL-6 and (B) IL-1β concentration at week 6 and 12 for subjects on *Labisia pumila* (white) or placebo (grey). Within-group comparisons were made using a *t*-test and * represents within group significance of P<0.05.

those on placebo (4.1 vs. 2.8, p=0.037). In contrast, women on placebo demonstrated significantly higher scores for "arousal" after 6 weeks of treatment versus women on *LP* (p=0.046); however, these scores were not maintained to week 12. When sexually active women were analyzed to determine whether the difference between *LP* and placebo was different between age groups (18-29, 30-39, 40-49 and ≥50 years), no significant interaction was found for age and total FSFI score, or any individual domain score after 6 and 12 weeks of treatment.

Safety evaluation

No serious AEs were reported during the study. The number of participants reporting AEs was similar in both groups (Table 5). In the placebo group, seven AEs with "possible relation to the treatment" were reported by four participants, while five AEs were reported by four participants in the *LP* group. All AEs except one resolved without any intervention. The other was menstrual cramping and resolved with concomitant medication. Vital signs, biometrics, hematological, clinical chemistry para-

Table 5. Adverse events with "possible" causal relation to the study medication.

Study group	Number of subjects	Adverse events "possibly" related to the medication listed for each subject
Labisia pumila	4	Nausea, menstrual cramps, vaginal spotting, mood alteration, stomach gas
Placebo	4	Breast tenderness, increased vaginal wetness, increased flatulence nausea, headache, mood alteration, constipation

meters and urinalysis were similar in both groups.

DISCUSSION

Sexual desire is a complex phenomenon that involves physiological and psychological influences and is thus difficult to treat (Jha and Thakar, 2010). Therapy options vary depending on the cause of sexual dysfunction, including the use of psychotherapy, prescription of estrogens, progestins or testosterone (Buster, 2013), but the gaps in the body of information related to female sexual dysfunction result in the condition remaining under-reported and poorly managed (Jha and Thakar, 2010). Extracts of *LP* have a history of use in South-East Asian women to maintain reproductive function and enhance sexual function (Melissa et al., 2012). To date, only one pilot human study has been published on the efficacy of water-soluble extracts of *LP* on menopausal symptoms, cardiovascular risk factors and hormonal profiles of Malay postmenopausal women (Kadir et al., 2012). The current study is the first to investigate the safety and efficacy of *LP* in a North American population of pre- and postmenopausal women.

This study recruited healthy women regardless of their level of sexual activity, as FSFI scores were not used as criteria for enrollment. Based on an FSFI cut off score of ≤26, only seven out of 36 enrolled participants were classified as sexually dysfunctional, and two were not sexually active for the duration of the study. As the majority of subjects in this trial were already sexually functional females, highlighting the effectiveness of *LP* in improving FSFI scores may have been confounded. Statistically significant results may have been more easily obtained if recruitment was limited to sexually active but sexually dysfunctional women (FSFI ≤ 26). Further, the absence of differences in FSFI scores between *LP* and placebo may be due to the wide age range of subjects. Subgroup analysis of FSFI scores on sexually active females showed that participants aged 30-39 years, 40-49 years and ≥50 years on herbal treatment performed better than females who were 18-29 years, though no definitive conclusions can be reached due to the small sample size.

Analysis of participants identified as sexually dysfunctional at baseline (FSFI ≤ 26) showed that women on *LP* reported significantly improved scores at week 6 in the "orgasm" domain versus those on the placebo; while higher scores continued to be reported at week 12 in women on *LP*, these did not reach significance. These results are consistent with literature as *LP* is reported to have aphrodisiac properties (Asiah et al., 2007). A bioactive peptide, recognized as an aphrodisiac marker, has also been identified in *L. pumila* (Asiah et al., 2007), perhaps eliciting improvements in FSFI scores. The total FSFI scores were improved in women identified with sexual dysfunction in both *LP* and placebo groups, though no definitive conclusions can be reached due to the small sample size.

The results of the self-reported SF-36 were not influenced by *LP* or placebo. Results from a previous study, in middle aged women, showed that while serious illness, employment and marital status were significant predictors of quality of life, hormone replacement therapy use and menopausal status were not (O'Dea et al., 1999). In a recent placebo-controlled double-blind study in Malaysian women between the ages of 40 and 60 years, a water extract of *LP* reduced anxiety levels by 55% compared to placebo (unpublished data). In the current study, the high baseline SF-36 and FSFI scores may have limited the response to the quality of life measures.

Water extracts of *LP* inhibit estradiol binding to antibodies against estradiol suggesting the presence of estrogen-like or estrogenic compounds (Husniza, 2000). Water extracts of *LP* were found to exhibit high estrogenic activity when tested in an *in vitro* alkaline phosphatase assay using Ishikawa cells and low induction of cell proliferation when compared against a positive estradiol control (Melissa et al., 2013). A study on Wistar rats found that treatment with either *LP* or estrogen replacement therapy had similar efficacy in preventing estrogen deficiency-induced changes from ovariectomy by regulating RANKL, OPG and BMP-2 gene expressions in femoral bones (Fathilah et al., 2013).

In the current study, 44% females on placebo consumed alcohol weekly in comparison to 17% on *LP*. Furthermore, 11% of females on *LP* were current smokers while none were smokers in the placebo group. As alcohol consumption increases circulating estrogen and androgen levels (Purohit, 1998), and smoking has anti-estrogenic effects (Tanko and Christiansen, 2004), these demographic differences may have impacted the results of the current study. Furthermore, participants on placebo had a significantly higher BMI and body weight at baseline in comparison to participants on *LP.* Increased

BMI, waist circumference and hip circumference are associated with increased levels of estrone, estradiol and free estradiol (Purohit, 1998), which may have influenced the results of the current study. Conclusions regarding the interaction between age and estradiol levels are difficult, since these parameters are dependent on the stage of the menstrual cycle at the time of blood sampling as well as the presence or absence of menstruation in the age groups. *LP* may be efficacious in older females deficient in female hormones; however, this was not specifically examined in this study. Previous data did not show significant fluctuations in follicle-stimulating hormone, luteinizing hormone and estradiol during a 280 mg/day intake of *LP* sprayed-dried water extract in 29 postmenopausal women versus placebo (Nik Hazlina et al., 2009b).

The link between cardiovascular disease (CVD) and sexual dysfunction in males is well-established; mild or moderate erectile dysfunction is much more common in patients with CVD (Archer et al., 2005). In contrast, sexual dysfunction in women with CVD has received limited attention (Steinke, 2010). Research has shown that the mechanism of clitoral engorgement is very similar to penile erection, and thus may also be adversely affected by CVD (Steinke, 2010). Neuropathy or vascular disease resulting from CVD risk factors such as hypertension, smoking, diabetes or hyperlipidemia are known organic causes to female sexual dysfunction (Archer et al., 2005).

Studies on rats showed a dose dependent decrease in TC with increasing doses of *LP* extract (unpublished). A significant reduction in trigly-cerides was also reported in post menopausal women on sprayed-dried water extract of *LP* for six months (Nik Hazlina et al., 2009a). 8-isoprostane is regarded as one of the best indices of lipid peroxidation and oxidative stress (Tanko and Christiansen, 2004). The decreasing trend in TC and urinary 8-isoprostane from baseline to week 12 seen in the current study, together with the literature, suggests a role for *LP* in decreasing CVD risk in women.

The analysis of renal and liver function tests, CBC and other clinical chemistry parameters showed that *L. pumila* was safe and well tolerated at a dose of 200 mg/day in the population studied. This is consistent with data from a six-month randomized placebo-controlled trial in postmenpausal women using dose regimens of up to 560 mg sprayed-dried water extract of *LP* (McTiernan et al., 2006; Nik Hazlina et al., 2009a; Nik Hazlina et al., 2009b).

The randomization schedule was designed to prevent an imbalance between groups with respect to menopausal status; therefore it is possible to suggest that the menopausal status of women did not affect the comparability of data between groups. As this was a pilot study, the purpose being to explore the effects of *LP* over a wide range of women, recruitment of subjects were not limited to a particular age-group or their sexual function.

An analysis of sexually active women on *LP* or placebo found no significant interaction between age and total FSFI scores, or any individual domain scores after 6 and 12 weeks of treatment. However, this may certainly be due to the small sample sizes of the subgroups. The small sample size limited the ability to stratify the population by age, BMI and menstrual status, and was certainly a limitation of this study and may have impacted the results. Identifying populations by sexual dysfunction defined by FSFI at recruitment as well as those sexually active may have provided better evaluation of the efficacy of *L. pumila* in improving sexual function. Lastly, the improvements in FSFI scores in the placebo group at week 6 that were not sustained through to week 12 could have been expected, as previous reports have suggested a placebo response in the treatment of sexual dysfunction (Bradford and Meston, 2009, 2011). A run-in period prior to enrollment in the study may have improved the response in FSFI scores by controlling for placebo effect.

Conclusions

This study investigates the efficacy of *LP* in healthy women regardless of their level of sexual function. While there were no significant differences between groups in the total FSFI score or domain scores for desire, arousal, lubrication, orgasm, satisfaction and pain at baseline or at 6 and 12 weeks of treatment, *LP* demonstrated a trend towards an improvement in TC, urinary 8-isoprostane and significant within group improvement in IL-6 and IL-1β suggesting a role for *LP* in improving inflammation. Future research of the effectiveness of *LP* on women's sexual well-being should focus on an older population of subjects that are sexually dysfunctional.

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