Herbal medicine for the treatment of oligo/amenorrhoea, hyperandrogenism and polycystic ovary syndrome (PCOS); a review of the laboratory and clinical evidence.

Polycystic ovary syndrome PCOS is a common reproductive and endocrine disorder affecting up to 17.8% of reproductive aged women [1]. It is a life-long condition and although the exact cause is yet to be identified, it is believed to have genetic origins, influenced by the uterine environment and behavioural factors [2]. PCOS presents with irregular menstruation and/or signs of hyperandrogenism [3]. Being overweight exacerbates all aspects due to underlying metabolic disturbance [4]. Serious long term health risks are associated including significantly increased risks for developing diabetes, cardiovascular disease, cancer and psychological morbidity including increased anxiety, depression and worsened quality of life [4]. Pharmaceutical treatments are only moderately effective at managing individual symptoms [4] and medical management places strong emphasis on a multidisciplinary approach [5].

The aim of this study was to critically review the evidence for effects of western herbal medicine in reproductive endocrinology and contribute to an understanding of the mechanisms for herbal medicines in oligo/amenorrhoea, hyperandrogenism and PCOS. We compared laboratory evidence including scientific studies using cell culture and animal models, with clinical data for proof-of-concept effects. We provide a narrative synthesis of the studies explaining in vitro and in vivo effects for herbal medicines with corroborating clinical evidence.
**Oligomenorrhoea and amenorrhoea**

Oligomenorrhoea is defined as menstrual cycle length that extends beyond 35 days (day one being the first day of menses). Amenorrhoea is defined as no menstrual period for three to six months or more, not due to pregnancy [2]. This review is focussed on hypothalamic, pituitary and ovarian causes with associated elevated gonadotropins including LH and prolactin and arrested folliculogenesis typically observed in polycystic ovaries. Hyperprolactinaemia is usually considered a unique cause for oligo/amenorrhoea, however in the present case it was included due to the potential co-existence for elevated prolactin, LH and PCOS, [6, 7].

**Hyperandrogenism**

Hyperandrogenism is defined as clinical or biochemically excessive androgens. Clinical markers in females include cutaneous manifestations such as the presence of acne, hirsutism and/or male pattern alopecia. Biochemical indications include elevated plasma concentration of androgens.

**Polycystic ovary syndrome (PCOS)**

According to the Rotterdam diagnostic criteria for PCOS, oligo/amenorrhoea and hyperandrogenism are two out of three features required for diagnosis [3]. The third feature of polycystic ovaries on ultrasound is not an essential requirement when the other two features are present [3, 8]. An associated endocrine feature for PCOS includes chronically elevated LH [9], which is strongly associated with infertility (p=0.0003) [7] and miscarriage [10]. Other endocrine features include elevated fasting glucose which is prevalent in approximately 31% of women with PCOS including normal weight women [11].

**Current management**
Pharmaceutical management includes the oral contraceptive pill (OCP) and ovulation induction with Clomiphene Citrate depending on fertility needs [12, 13]. Women with PCOS are however likely to exhibit contraindications for the OCP [4] and whilst induction of ovulation with clomiphene has demonstrated success, pregnancy rates remain inexplicably low [14]. Up to thirty 30% of women, particularly overweight women with PCOS, fail to respond to clomiphene therapy [14-16].

Herbal medicines are known to contain pharmacologically active constituents with physiological effects on female endocrinology and have been positively associated with reduced incidences of breast cancer, osteoporosis and cardiovascular disease [17-22]. Herbal medicines are complex interventions with the potential for synergistic and antagonistic interactions between compounds [23].

Methods

We undertook two separate searches. The first (laboratory) search sought data explaining the effects of herbal medicine on reproductive endocrinology; the second (clinical) search sought clinical data corroborating the effects of herbal medicine in oligo/amenorrhoea, hyperandrogenism and PCOS.

We searched the following electronic databases: the Cochrane Library, MEDLINE (1950 to present), MEDLINE ovidSP, CINAHL (1936 to present), SciVerse, EBSCO, EMBASE, ProQuest, PubMed, from the date of database inception to June 2014. In addition, we manually searched bibliographies of review articles. Key terms included: herbal medicine, herbal extracts, phytotherapy AND androgens, oestrogens, follicle stimulating hormone, luteinising hormone, prolactin, insulin, glucose and polycystic ovaries. Search terms for clinical trials included the following key words; menstrual irregularity, oligomenorrhoea, amenorrhoea,
hyperandrogenism, hirsutism, acne, polycystic ovary syndrome (PCOS) polycystic ovaries, oligo-ovulation, anovulation, fertility, infertility and pregnancy AND six herbal medicines identified from the laboratory search (Vitex agnus-castus, Cimicifuga racemosa, Tribulus terrestris, Glycyrrhiza glabra, Paeonia lactiflora and Cinnamomum cassia).

Our laboratory search included investigations into the effects of herbal medicine using computer models, cell cultures, animals with PCOS induced with oestradiol valerate and androgens and sterilised and ovariectomised rats. We included studies which commenced with a whole herbal extracts with extended investigations into fractionated components isolated during the study. We excluded laboratory studies which commenced using isolated chemicals not directly extracted from crude herbal medicines, such as biguanide pharmaceuticals [24], and studies examining androgen effects in male animals.

Our second search for clinical trials was performed without language restriction and included randomised controlled trials and single arm clinical trials. We included clinical studies investigating commercially available herbal extracts and investigations that compared the effectiveness of herbal medicine with pharmaceuticals. We excluded clinical studies investigating herbal medicines with unrelated physiological outcomes.

Results

Laboratory studies

Our search identified 33 laboratory (pre-clinical) studies. Eighteen studies met the inclusion criteria, nine reported on receptor binding assays or ovarian or pituitary (brain) cell cultures, [25-33] and nine used an animal experimental model with hormone assays and/or post-
mortem examination of ovarian, uterine and brain histology, [34-42]. We excluded 15 studies for the following reasons; investigation of CHM formula without explained reproductive endocrine effects (n=6), investigation of herbal medicine for alternative outcomes (n=6) and investigations which commenced with isolated constituents (n=3).

Clinical studies

Forty five clinical studies were identified for inclusion/exclusion assessment. A pre-requisite for the inclusion of clinical studies was previously identified laboratory evidence explaining the mechanism of effect in reproductive endocrinology. Fourteen met the inclusion criteria [43-56]. Seven were randomised controlled trials (RCT’s) including 568 women [50-56]. Thirty one were excluded for the following reasons; investigation of herbal formulations containing multiple herbs without pre-clinical evidence of effects for the formulation (n=9), conditions different to those specified (n=16), investigation of isolated herbal chemicals (n=3) and herbal medicines without laboratory evidence explaining the mechanism of effect (n=3).

Six RCT’s examined commercially produced herbal medicine extracts. These were Vitex *agnus-castus* in the form of Strontan®, Mastodynon®, Phyto Hypophyson® and Agnacaston® and *Cimicifuga racemosa* in the form of Klimadynon®[54, 56].

Please refer to Tables 1 and 2 in separate document: ARENTZ et al Tables 1 and 2.doc

*Herbal medicines with effects in oligo/amenorrhoea, hyperandrogenism and PCOS*

**Vitex agnus-castus**

Pre-clinical and clinical evidence was found for *Vitex agnus-castus* for lowering prolactin and improving menstrual regularity and treating infertility. *V. agnus-castus* contains a variety of
compounds which bind to dopamine type 2 (DA-2) receptors in the brain; reduce cAMP and lower prolactin secretion (Table 1). This was demonstrated in studies using recombinant DA-2 receptor proteins, and basal and stimulated rat pituitary cell cultures [27-30]. Prolactin lowering effects were found for normal and ovariectomised rats [38]. Additional agonistic opiate effects were observed in studies using human opiate receptors cell cultures [57]. Clinical equivalence for prolactin lowering effects of *V. agnus-castus* and the pharmaceutical Bromocriptine was found in one study [52]. Three RCT’s demonstrated positive effects for *Vitex agnus-castus* in oligo/amenorrhoea and infertility [50, 51, 53]. Methodological shortcomings included not reporting baseline characteristics for subgroups and small sample sizes however clinical outcomes demonstrated physiological effects consistent with laboratory and animal findings. (Table 1 and 2).

*Cimicifuga racemosa* was found to lower LH in two laboratory studies both examining cell cultures from ovariectomised rats [34, 41]. The mechanism occurred through competitive inhibition of oestrogen following the selective binding of oestrogen receptors (ERα) on the hypothalamus and pituitary [41]. An earlier study found contrary results for reduction of LH, however this study investigated an isolated flavonoid and suggested that other constituents may be active [26]. Two RCT’s corroborate the positive fertility effects for *Cimicifuga racemosa* in women with PCOS, used in conjunction and when compared with the pharmaceutical *Clomiphene citrate*, [54, 58] These two RCT’s report results for over200 women. Findings concur with laboratory and animal studies; however potential risks for bias include baseline imbalance and attrition bias. (Table 2).

*Tribulus terrestris*

Two laboratory based RCT’s examined the effects of *Tribulus Terrestris* in rats with
polycystic ovaries induced with oestradiol valerate [35, 36]. (Table 1). Both studies demonstrate significantly improved ovulation rates for animals treated with two doses of \textit{T. terrestris} extracts compared to controls. Although the endocrinological effects are not described in either study, laboratory findings of ovulation induction are supported by the clinical findings of elevated FSH following treatment with \textit{T. terrestris} [45].

In a prospective, observational clinical trial examining the endocrine effects of \textit{T. terrestris} extract 750mg per day, over five days in 8 healthy women (aged 28-45), a significant increase for mean serum FSH concentration from 11mIU/ml before treatment to 17.75mIU/ml following treatment (P<0.001) was demonstrated. Pre-treatment FSH levels returned following cessation of treatment. Another clinical study evaluated the equivalence of \textit{T. terrestris} (Tribestan®) and pharmaceuticals for ovulation induction in women with oligo/anovular infertility (n=148), [49]. During the three month follow up, ovulation rates were highest with \textit{Epimestrol} (74%), followed by \textit{T. terrestris} (60%), \textit{Clomiphene citrate} (47%) and \textit{Cyclofenil} (24%). However, the evidence for \textit{T. terrestris} should be interpreted with caution due to risks for bias in clinical studies. One study was uncontrolled with a small number of healthy participants [45], the second study did not report baseline characteristics, methods for allocation to treatment groups and data were not statistically analysed [49].

\textit{Glycyrrhiza glabra}

Androgen lowering effects for \textit{Glycyrrhiza glabra} have been demonstrated in one laboratory study examining hormone concentration in female rats, [42] and corroborated in two clinical trials, one including healthy women [44] and the other including women with PCOS [43]. The animal study reported significantly reduced free and total testosterone and
increased oestradiol in sterilised rats and no hormonal changes in oophrectomised rats. The authors conclude that the hormonal effects occurred primarily in the ovary via enhanced aromatisation of testosterone to 17-beta oestradiol. The investigators also observed significantly increased oestradiol. There were no changes to FSH or LH in androgen sterilised or oophrectomised rats [42].

Another animal study examined the effects of *G. glabra* on the morphological features of polycystic ovaries using immunohistochemistry [39]. This study demonstrated significantly increased ovulation rates by the number of corpus luteum in polycystic ovaries compared with controls. The authors propose that the mechanism of effect for *G. glabra* was competitive inhibition of oestrogen at oestrogen receptor sites, limiting the production of nerve growth factor (NGF), its neurotropic effects and inhibition of sympathetic neurological involvement in the pathogenesis of polycystic ovaries.

Two clinical studies examined the androgen lowering effects of *G. Glabra*. A single arm clinical trial demonstrated reduced testosterone in healthy women aged 22-26 years (n=9) over two menstrual cycles. Treatment with *G. glabra*, 7 grams per day reduced testosterone from 27.8±8.2 to 17.5 ±6.4, p<0.05 [44]. Another single arm clinical trial investigated the effects of *G. glabra* in women with PCOS, (n=32). *G. glabra* 3.5g per day was added to anti-androgen pharmaceutical treatment, Spirinolactone 100mg/day over two menstrual cycles. An unwanted side effect for Spirinolactone was the flare of androgens during the initial phase of treatment. This study demonstrated reduced concentrations of testosterone during the first four days of treatment at 103±29ng/d in the Spirinolactone group compared to 91 ng/d ±19 when combined with G. glabra (p<0.05) [43]. Consistent laboratory and clinical outcomes were demonstrated however limitations included design shortcomings. Both clinical studies were open label observational design with small sample sizes; one
included healthy participants. Rigorous studies are needed to confirm the androgen lowering effects of \textit{G. glabra} in hyperandrogenism and PCOS.

Results for \textit{G. glabra} (and indeed any herbal ingredient) were complicated in this case by the variation in herbal extraction processes and the concomitant variability in chemical profiles of the herbal ingredients. The laboratory studies of the herbal material were based on crude aqueous extracts whilst the clinical studies were based on ethanol extracts. Despite variability in the herbal extraction methods, both laboratory and clinical studies demonstrated anti-androgenic effects.

\textit{Paeonia lactiflora} and \textit{Glycyrrhiza glabra}

One laboratory study and two clinical investigations provide indicative evidence for the two herb combination, \textit{Glycyrrhiza glabra} and \textit{Paeonia lactiflora} \cite{42, 47, 48}. An animal study found significant reductions in free and total testosterone following exposure to the combination \cite{42}. (Table 1). These findings are supported in two open label clinical trials including women with PCOS (n=34) \cite{48} and women with hyperandrogenism (n=8) \cite{47}. Both trials examined the effects on androgens for the aqueous extract TJ-68 (equal parts \textit{G. glabra} and \textit{P. lactiflora}), 75 grams per day for 24 weeks and 5-10 grams per day for 2-8 weeks respectively. In the trial including women with PCOS, mean serum testosterone was significantly reduced from 137.1 ng/dL (± 27.6) to 85.3 ng/dL (± 38), \textit{p}<0.001 at four weeks of treatment \cite{48}. Similar effects were observed in the women with oligomenorrhoea and hyperandrogenism which showed serum testosterone reduced from 50-160ng/dL prior to treatment to less than 50ng/dL \cite{47}. However statistical significance was not reached due to the small sample size despite positive outcomes in seven out of eight participants.
Paeonia lactiflora and Cinnamomum cassia

Paeonia lactiflora combined with Cinnamomum cassia in a preparation called Unkei-to was investigated in an in-vitro study for ovarian production of 17-beta-oestradiol and progesterone, [31]. Granulosa cells obtained from women undergoing IVF were examined for steroid hormone concentration following incubation with different doses over 48 hours. Oestradiol was significantly increased (p<0.01) following exposure to doses of 0.3ug/ml of Unkei-to. Supporting clinical evidence was found in one clinical trial of 157 infertile women aged 17-29 years, including a subgroup of 42 women with hyper-functioning (PCOS) oligo/amenorrhoea. Treatment with Unkei-to, 7.5 grams per day for eight weeks, demonstrated significant reductions of mean LH in the PCOS sub-group of 49.7% (±15.3). Ovulation was confirmed in 30 out of 42 oligo/amenorrheic women [46]. Limitations however include findings based on sub-group comparisons without description of subgroup baseline characteristics (other than oligomenorrhoea). Although the same aqueous extract intervention was investigated in pre-clinical and clinical studies, it contained additional herbal extracts and it was irrational to attribute hormonal effects to P. lactiflora and C. cassia.

Cinnamomum cassia

An animal study compared the effectiveness of C. cassia and the pharmaceutical Metformin on hormone concentration in rats with PCOS [37]. Both interventions demonstrated significant improvements compared to controls at 15 days for measures of testosterone ng/ml (control 0.747 ±0.039; metformin 0.647 ± 0.027; C. cassia 0.625±0.029); LH ng/ml (control 7.641±0.267; metformin 6.873 ± 0.214; C. cassia 6.891 ± 0.221) and insulin resistance (HOMA-IR) (control 10.018 ± 0.217; metformin 7.067 ± 0.184 C. cassia 8.772 ±
The metabolic effects for *C. cassia* were further demonstrated in overweight women with oligo/amenorrhoea and PCOS in a placebo controlled RCT (Table 2), [55]. However, although the RCT had low risks for bias it was a pilot study primarily investigating feasibility. Outcomes were promising for metabolic profile in PCOS however the sample size was small and the authors recommended further studies.

### Summary of results

This review includes 18 preclinical laboratory based studies and 14 clinical trials. We found reproductive endocrine effects in oligo/amenorrhoea, hyperandrogenism and/or PCOS for six herbal medicines. The quality of evidence, as determined by the volume of pre-clinical studies and the methodological quality of clinical trials, was highest for the herbal medicines *Vitex agnus-castus*, *Cimicifuga racemosa* and *Cinnamon cassia*, for which there were laboratory and/or animal studies demonstrating endocrine mechanisms of action consistent with clinical outcomes shown in RCT’s with low risks for bias. However, replicated RCT data was only found for one herbal medicine, *C. racemosa*.

Evidence for *Tribulus terrestris*, *Glycyrrhiza glabra* alone and in combination with *Paeonia lactiflora* and *P. lactiflora* with *Cinnamomum cassia* was limited by the volume of laboratory and animal studies, with only one to two studies found for each herb or herbal combination. There was supporting clinical data however many were small single arm, open label studies measuring endocrine effects in healthy women. Evidence for these herbal medicines is preliminary and in an emergent phase.

### Discussion
This review synthesises the evidence for mechanisms of effect for herbal medicine in oligo/amenorrhea, hyperandrogenism and PCOS. Laboratory, animal and clinical studies demonstrate that the herbal medicines Vitex agnus-castus, Cimicifuga racemosa and Tribulus terrestris initiate endocrine effects in the pituitary as measured by lowered prolactin and LH and raised FSH. Four herbal medicines, Tribulus terrestris, Glycyrrhiza glabra, (alone and in combination with Paeonia lactiflora), Paeonia lactiflora (in combination with Cinnamomum cassia) and Cinnamomum cassia demonstrated morphological changes in polycystic ovaries and steroidogenesis, including reduced ovarian volume and cysts, lowered androgens, improved insulin sensitivity and increased oestradiol.

Herbal medicine may present a treatment option for women with oligo/amenorrhea, hyperandrogenism and PCOS as an adjunct or alternative treatment to pharmaceuticals with a high degree of acceptability by women with PCOS [59]. Preliminary evidence for equivalent treatment effects were found for the pharmaceuticals Bromocriptine, in the management of hyperprolactinaemia (V. agnus-castus) and for Clomiphene citrate for infertility and ovulation induction (C. racemosa and T. terrestris). Herbal medicine had positive adjunct effects with the pharmaceuticals Spirinolactone in the management of hyperandrogenism (G. glabra), and clomiphene for PCOS related infertility (C. racemosa). It is important however to highlight that evidence was provided by a limited number of clinical studies, some with significant risks for bias; particularly Tribulus terrestris, Glycyrrhiza glabra alone and in combination with Paeonia lactiflora and P. lactiflora in combination with Cinnamomum cassia.

Complementary medicine use by women has increased in western countries during the past 10 years [60-64], with rates of use ranging between 26% and 91% [61, 62]. One of the
popular types of CM is herbal medicine. Herbal medicines prescriptions for oligo/amenorrhea, hyperandrogenism and PCOS usually include the combination of *Glycyrrhiza glabra* and *Paeonia lactiflora* [65, 66]. We found preliminary evidence for this combination for hyperandrogenism only, and the evidence was more robust for *G. glabra* alone than when combined with *P. lactiflora*. Comparatively, our findings for the combination of *P. lactiflora* and *C. cassia* demonstrated no change in androgen concentration, suggesting that the anti-androgen activity in the *G. glabra* combination was more likely attributable to *G. glabra* rather than *P. lactiflora*. However, our findings may be complicated by the aqueous extraction methods used in the *P. lactiflora* and *C. cassia* combination. More research into the anti-androgen effects of the combination *G. glabra* and *P. lactiflora* is needed to clarify the anti-androgen mechanism particularly if this herbal combination remains cornerstone herbal management for PCOS.

This review has some limitations. Our inclusion criteria for clinical studies relied upon our identification of preclinical evidence describing the mechanisms of effect for herbal medicines; clinical studies were excluded from this review due to the absence of mechanistic evidence. We used a deductively logical approach to assess clinical outcomes which is not consistent with traditional or individualistic rationale for herbal selection. This study was intended to inform the physiological effects of herbal medicine in oligo/amenorrhoea, hyperandrogenism and PCOS and add to consumers and clinicians understanding for the strength of evidence for herbal medicine for these common conditions.
Conclusions

Preclinical and clinical studies provide preliminary evidence that six herbal medicines may have beneficial effects for women with oligo/amenorrhea, hyperandrogenism and PCOS. The quality of the evidence is variable and is strongest for *Vitex agnus-castus* and *Cimicifuga racemosa* in the management of oligo/amenorrhea and infertility associated with PCOS; and *Cinnamomum cassia* for improving metabolic hormones in PCOS. Evidence for *Tribulus terrestris*, *Glycyrrhiza glabra* alone and in combination with *Paeonia lactiflora* and *P. lactiflora* combined with *Cinnamon cassia* is promising but in an emergent phase. Larger well-designed studies for herbal medicines for these common conditions are needed.


3. ESHRE. Revised 2003 consensus on diagnostic criteria and long-term health risks associated with polycystic ovary syndrome, Fertility & Sterility, 2004. 81(1).


37. Heibashy, M., G. Mazen, and M. Shahnin, Metabolic Changes and Hormonal Disturbances in Polycystic Ovarian


42. Takeuchi, T.N., Osamu; Okamura, Takashi; Yangui, Tsutomu. Effect of traditional herbal medicine, shakuyaku-kanzo-to on total and free serum testosterone levels. The American journal of Chinese medicine, 1989. 17(01n02): p. 35-44.


Additional files provided with this submission:

Additional file 1: ARENTZ et al. Tables 1 and 2.docx, 48K
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