

Full Length Research Paper

The safety, efficacy, and quality of *Trifolium pratense* (Red clover) for menopause relief

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The objective of this review was to evaluate the safety, efficacy, and quality of red clover (*Trifolium pratense*) for the relief of menopausal symptoms, and to assess its potential as an alternative or adjunct to conventional hormone replacement therapy (HRT). A scoping review was conducted, including randomized controlled trials, observational studies, and systematic reviews published between 2000 and 2025 that assessed the safety, efficacy, or quality of *T. pratense* in peri- or postmenopausal women. Data were extracted on menopausal outcomes, adverse events, drug interactions, and product quality, including isoflavone standardization and potential contaminants. More than 30 human clinical trials and systematic reviews were identified. Efficacy was most consistent for vasomotor symptoms, with ≥80 mg/day of standardized isoflavones reducing both the frequency and severity of hot flushes. Significant improvements were also reported for vaginal dryness, atrophy, and sexual function, whereas evidence for sleep disturbances and urinary symptoms was inconsistent. Safety was generally favorable over durations ranging from 8 weeks to 3 years, with no significant effects on coagulation or CYP450 activity. Rare adverse events were primarily linked to adulteration rather than intrinsic toxicity. Quality assessments revealed substantial variability in isoflavone content and contamination risks across commercial products. Standardized *T. pratense* appears safe and moderately effective for selected menopausal symptoms. However, variability in commercial product quality, limited long-term safety data, and a scarcity of herb–drug interaction trials restrict confident clinical application. Standardization using Aglycone Isoflavone Equivalents (AIE) and long-term randomized controlled trials (RCTs) across diverse populations are needed to confirm its therapeutic potential.

Key words: Menopause, isoflavones, *Trifolium pratense*, phytoestrogens, safety, quality control.

INTRODUCTION

Menopause is defined as the permanent cessation of menstruation for 12 consecutive months (Gatenby and Simpson, 2024), typically occurring between the ages of 45 and 55 due to declining ovarian oestrogen production (Gatenby and Simpson, 2024; Santoro et al., 2021). This hormonal decline leads to symptoms that may

substantially impair quality of life, including vasomotor symptoms (hot flushes, night sweats); sleep disturbances, mood changes, vaginal dryness, and cognitive alterations (Daan and Fauser, 2015; Santoro et al., 2021). Reduced oestrogen levels also contribute to important long-term health risks. Bone loss accelerates due to decreased

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bone mineral density, increasing the risk of osteoporosis and fractures (Yong and Logan, 2021). Loss of cardioprotective effects elevates the risk of cardiovascular disease (Lee et al., 2022). Cognitive and emotional symptoms, such as memory lapses and mood fluctuations, may also occur (Santoro et al., 2021).

The benefits and risks of hormone replacement therapy (HRT)

HRT remains the most effective treatment for menopausal symptoms and for the prevention of long-term consequences associated with oestrogen deficiency. It involves administering oestrogen alone or in combination with progestogens to counter declining hormone levels (Cameron et al., 2024). Many commonly used compounds are synthetic derivatives designed for improved bioavailability, receptor specificity, and metabolic stability (Stahlberg et al., 2003). Oestrogen formulations include conjugated equine oestrogens and ethinyl oestradiol, the latter containing a C17 ethinyl modification that enhances oral potency (Stahlberg et al., 2003). Progestins, such as medroxyprogesterone acetate (MPA) and norethisterone, feature structural modifications that increase potency and oral activity (Stahlberg et al., 2003). Contemporary HRT focuses on personalized regimens based on age, symptom severity, and comorbid risk factors (Lobo, 2017). HRT reduces vasomotor symptoms by 75 to 80% (Cagnacci and Venier, 2019), prevents genitourinary syndrome, lowers fracture risk by 30 to 40%, and may provide cardiovascular protection when initiated before 60 years of age or within 10 years of menopause (Cameron et al., 2024; Lobo, 2017).

Safety concerns continue to influence clinical decision-making. Women's Health Initiative (WHI) follow-up data indicate that oestrogen-only therapy reduces breast cancer incidence by 21%, whereas combined therapy increases it by 29% (Chlebowski et al., 2020). Risks of stroke and venous thromboembolism also exist but are lower in healthy, younger postmenopausal women (Tao et al., 2021; Zaw et al., 2018), and the absolute risk remains small approximately eight additional invasive breast cancer cases per 10,000 women per year of combined HRT use (Chlebowski et al., 2020).

Following the initial WHI publication, HRT prescribing declined but has risen by roughly 30% since 2015 due to improved risk stratification and updated guidance (Cameron et al., 2024). Concerns regarding HRT safety have stimulated interest in alternatives, such as selective serotonin reuptake inhibitors/serotonin–norepinephrine reuptake inhibitors (SSRIs/SNRIs), gabapentinoids, clonidine, oxybutynin, selective estrogen receptor modulators (SERMs), micronized hormones, and adjusted hormonal regimens (Lobo, 2017; Santoro et al., 2021; Goyal et al., 2025). Botanical therapies rich in phytoestrogens, such as *Trifolium pratense*, are also

widely used (Ghazanfarpour et al., 2016).

Despite being perceived as “natural,” botanical supplements can exert clinically relevant physiological and pharmacological effects and may interact with conventional medicines, highlighting the need for routine screening during clinical evaluations and pre-surgical assessments.

Background literature analysis and critique

Chemical composition of *T. pratense*

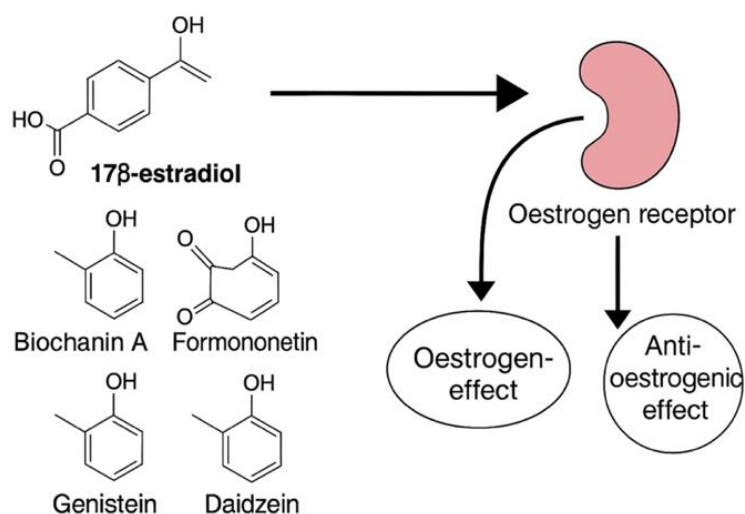
T. pratense is a Fabaceae species native to Europe, Northwest Africa, and Western Asia (Booth et al., 2006a). Traditional medicine systems have utilized the aerial parts (stems, leaves, and flowers) for various conditions, including respiratory and dermatological ailments, fever, asthma, and whooping cough, as well as for topical treatment of eczema, rashes, psoriasis, and wound healing (Nelsen et al., 2002; Gikas et al., 2008). These long-standing uses reflect its historical role as a multipurpose botanical remedy across Europe, India, Central Asia, and North Africa. Its therapeutic properties are primarily attributed to its rich isoflavone content plant-derived phytoestrogens structurally similar to 17 β -oestradiol, with comparable phenolic ring features that enable weak oestrogen-receptor binding (Gómez-Zorita et al., 2020). Depending on the physiological context, isoflavones can exert either oestrogenic or anti-oestrogenic effects (Gómez-Zorita et al., 2020). The primary isoflavones found in *T. pratense* include biochanin A (5,7-dihydroxy-4'-methoxy-isoflavone), formononetin (7-hydroxy-4'-methoxy-isoflavone), genistein (5,7,4'-trihydroxy-isoflavone), and daidzein (7,4'-dihydroxy-isoflavone). Figure 1 shows the chemical structures of the major isoflavones found in *T. pratense* (Biochanin A, Formononetin, Genistein, and Daidzein), highlighting their structural similarities to 17 β -oestradiol and their potential interactions with estrogen receptors. Table 1 shows the key properties of the primary isoflavones found in *T. pratense* and their structural features.

Biochanin A and formononetin are particularly significant, as they are metabolized to genistein and daidzein, respectively, which possess greater estrogenic activity (Booth et al., 2006b). *T. pratense* contains up to 30 times more total isoflavones than soybeans (Papadopoulos et al., 2006). The minimum effective daily dose is approximately 40 mg, which is commonly included in standardized supplements (Fiala and Pospisiil, 2012), while doses up to 120 mg/day have demonstrated strong safety (Fiala and Pospisiil, 2012). Most clinical trials used doses ranging from 40 to 160 mg/day, with 80 mg/day showing the most consistent efficacy for vasomotor symptoms (Hidalgo et al., 2005; Ghazanfarpour et al., 2016).

Table 1. Key properties of the primary isoflavones found in *T. pratense* and their structural features.

Isoflavone	Structure	Key properties
		Precursor to genistein
<i>Biochanin A</i>		Biochanin A is a methylated isoflavone that is a dominant compound in <i>T. pratense</i> leaves (Lipovac et al., 2015)
<i>Formononetin</i>		Formononetin undergoes demethylation to produce daidzein, which peaks in serum after about 12.6 h (Lipovac et al., 2015)
<i>Genistein</i>		Potent ER β agonist
<i>Daidzein</i>		Converted into equol (a non-steroidal oestrogen) by gut microbiota (Schröder et al., 2013).

Chemical structures were retrieved from PubChem (2004); PubChem Compound IDs: genistein, CID 5280961; biochanin A, CID 5280373; formononetin, CID 5280378; daidzein, CID 5281708).

**Figure 1.** The chemical structures of major isoflavones found in *T. pratense* (Biochanin A, Formononetin, Genistein, and Daidzein) highlighting their structural similarities to 17 β -oestradiol and their interaction with oestrogen receptors.

Source: Self-generated from Ghazanfarpour et al. (2015).

Comparison with other natural remedies for menopause

Compared with black cohosh (*Actaea racemosa*), which likely acts through serotonergic and dopaminergic modulation rather than oestrogenic activity (Powell et al., 2008), *T. pratense* contains phytoestrogens with established receptor binding (Chen et al., 2015). Soy (*Glycine max*) contains genistein and daidzein, whereas *T. pratense* contains their precursors, biochanin A and formononetin (Geller and Studee, 2005; Andres et al., 2015). These precursors may yield longer-lasting or more variable effects depending on gut microbiota composition (Schröder et al., 2013; Matthies et al., 2008). Meta-analyses suggest that *T. pratense* may offer more consistent vasomotor benefits than soy, while black cohosh may be more beneficial for psychological symptoms but has been linked to potential hepatotoxicity (Mahady et al., 2006). However, head-to-head trials are scarce, and methodological differences limit direct comparisons.

METHODOLOGY

Study design

This scoping review mapped and synthesized evidence on the safety, efficacy, and quality of *T. pratense* for the relief of menopausal symptoms. The review was conducted following the PRISMA-ScR 2020 guidelines and methodological principles outlined in the Cochrane Handbook for Systematic Reviews of Interventions.

Inclusion criteria

Studies were included if they:

- i) Examined peri- or postmenopausal women,
- ii) Investigated *T. pratense* in any form (standardised extract, isolated isoflavones, dried plant material, or multi-component preparations).
- iii) Used placebo, HRT, or active comparators,
- iv) Reported outcomes relating to symptom relief, safety/tolerability, pharmacokinetics, interaction potential, or product quality,
- v) Employed RCTs, non-randomised studies, observational designs, pharmacokinetic studies, systematic reviews, or meta-analyses,
- vi) Were published in peer-reviewed journals between 2000–2025.

Exclusion criteria

Studies were excluded if they:

- i) Used only animal models without relevance to human pharmacodynamics/safety,
- ii) Were unavailable in English without accessible translation.

Information sources and search strategy

A comprehensive search was conducted in PubMed/MEDLINE, ScienceDirect, Scopus, Embase, and the Cochrane Library.

Additional studies were identified through Google Scholar and manual reference screening. Search terms included botanical identifiers (*T. pratense*, red clover), menopause-related clinical concepts, safety terms (adverse effects, interactions), pharmacological descriptors (dose–response, pharmacokinetics), and supplement-quality terms (isoflavones, dietary supplements, quality control).

Screening and selection process

All citations were imported into EndNote 21 for deduplication. Titles, abstracts, and full texts were screened independently by the reviewer (CVN). Full-text eligibility was assessed following PRISMA 2020 criteria (Page et al., 2021). Any uncertainties were resolved through discussion with the project supervisor.

Data extraction and charting

Data from included studies were extracted into a standardized form capturing:

- i) Study characteristics (author, year, design, sample size, duration),
- ii) Population details (age, menopausal status, inclusion/exclusion criteria),
- iii) Intervention details (formulation, dosage, duration),
- iv) Comparators (placebo, HRT, active agents),
- v) Outcomes (efficacy endpoints, safety/adverse events, quality measures),
- vi) Key findings and statistical significance (effect sizes, p-values, and confidence intervals where available).

Data synthesis and analysis

A narrative synthesis was conducted, grouping findings into three domains: efficacy, safety, and quality. Results were stratified by symptom category, including vasomotor, sleep, urogenital, psychological, and bone health outcomes. The strength of evidence (high, moderate, or low) was determined based on the number, consistency, and quality of RCTs. PRISMA principles guided transparent reporting throughout the review. Figure 2 shows the PRISMA 2020 flow selection process for the scoping review on *T. pratense* for menopause symptom relief (Page et al., 2021).

RESULTS AND DISCUSSION

Efficacy in alleviating menopausal symptoms

Vasomotor symptoms

Vasomotor symptoms affect 75 to 80% of menopausal women and substantially impair quality of life (Hamoda and Sharma, 2024). Multiple clinical trials have assessed the effect of *T. pratense* on hot flashes and night sweats. A meta-analysis by Ghazanfarpour et al. (2015) showed reduced hot flash frequency, particularly in women experiencing ≥ 5 hot flashes per day, with a mean difference (MD) of -1.99 ($p = 0.067$), although heterogeneity was high ($I^2 = 94.93\%$). In a randomized, placebo-controlled study of 72 postmenopausal women, Shakeri et al. (2015) reported significant reductions in Menopause Rating Scale (MRS) scores after 12 weeks of

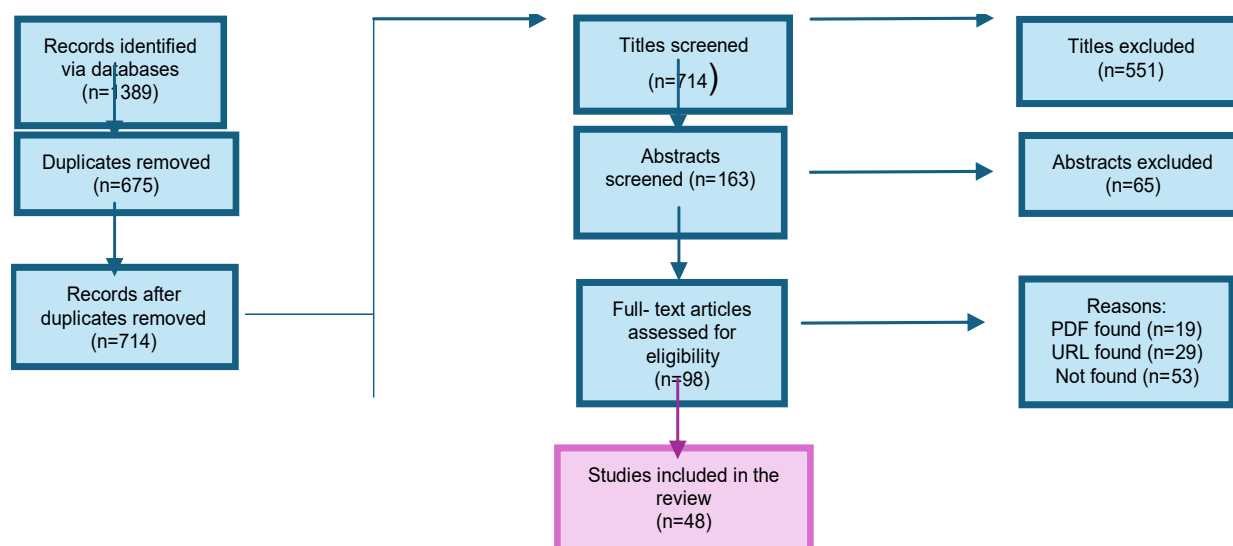


Figure 2. PRISMA 2020 flow diagram illustrating the study selection process for the scoping review on *T. pratense* for menopause symptom relief.
Source: Page et al. (2021).

dried *T. pratense* leaves (40 mg twice daily), from 20.41 to 10.08 ($p = 0.0001$), compared with a smaller decrease in the placebo group (20.77 to 17.20). Improvements primarily reflected reductions in somatic, vegetative, and psychological symptoms. A meta-analysis by Kanadys et al. (2021) also found significant reductions in daily hot flashes with *T. pratense* (weighted MD = -1.73).

Benefits were greatest in women experiencing ≥ 5 daily hot flashes and when isoflavone doses exceeded 80 mg/day or formulations were high in biochanin A. Lipovac et al. (2012) conducted a randomized, double-blind crossover study in 109 postmenopausal women using 80 mg/day standardized isoflavones for 90 days. Significant reductions were observed across vasomotor (73.5%), somatic (72.2%), and psychological (75.4%) symptoms ($p = 0.0001$), compared with minimal placebo effects (8.2%, 0.9%, and 6.7%). Symptom improvements reversed during washout and placebo phases, and no adverse events were reported. Similarly, Hidalgo et al. (2005) found that 80 mg/day *T. pratense* significantly improved Kupperman Index (KI) scores ($27.2 \rightarrow 5.9$), with smaller improvements in the placebo phase (mean 20.9), alongside enhanced vaginal epithelial maturation. A modest improvement in lipid profiles was noted, although not consistently significant. Overall, the evidence consistently supports *T. pratense* (≥ 80 mg/day isoflavones) in reducing vasomotor symptoms, particularly in women with more frequent hot flashes.

Sleep disturbances

Sleep disturbances affect 40 to 60% of menopausal women, often related to vasomotor instability (Gatenby

and Simpson, 2024; Thomas et al., 2014). Only two clinical trials specifically evaluated sleep outcomes with *T. pratense*. In a 12-week trial using the Pittsburgh Sleep Quality Index (PSQI), Geller et al. (2009) observed modest, non-significant improvement in the “hot flash impact on sleep” subscale (MD = -0.45 ; $p = 0.33$). Overall PSQI sleep scores were slightly poorer in the *T. pratense* group (MD = 0.27; $p = 0.33$), although the PSQI subscale represented only a small component of the overall score and may not fully capture vasomotor-related sleep changes. A randomized crossover trial by Hidalgo et al. (2005) reported more notable improvements: sleep-related complaints declined from 86.8% to 41.5% in the *T. pratense* group, compared with a smaller decrease from 86.8% to 73.3% during placebo. These findings suggest potential benefit where sleep disruption is secondary to hot flashes. Overall, evidence for sleep outcomes remains mixed but indicates that *T. pratense* may improve sleep when disturbances are linked to vasomotor symptoms.

Urogenital symptoms

Urogenital symptoms, such as vaginal dryness, atrophy, and dyspareunia, are common during menopause due to declining oestrogen levels, and several studies report improvements with *T. pratense* supplementation. In a double-blind crossover trial, Hidalgo et al. (2005) found that an 80 mg/day standardized isoflavone extract significantly enhanced vaginal epithelial maturation compared with placebo, indicating a direct estrogenic effect on urogenital tissue. This improvement aligned with reductions in Kupperman Index scores, suggesting

symptom relief across both local and systemic menopausal domains. Additional studies by Khayatan et al. (2019) similarly reported increased vaginal moisture, reduced discomfort during intercourse and improved overall sexual function following *T. pratense* use, and although these outcomes were often secondary endpoints and sample sizes were relatively small. Differences in extract composition and treatment duration also contribute to variability in response. Nonetheless, consistent trends across trials suggest that standardized *T. pratense* preparations may offer meaningful support for women experiencing vulvovaginal atrophy, particularly at doses of ≥ 80 mg/day of isoflavones. Overall, the evidence indicates a moderate but consistent benefit of *T. pratense* for urogenital symptoms, especially with well-standardized extracts.

Psychological symptoms

Psychological symptoms, including anxiety, irritability, mood swings, and emotional instability, frequently accompany the menopausal transition. Several clinical trials demonstrate that *T. pratense* may alleviate these symptoms, particularly when they occur alongside vasomotor disturbances. Shakeri et al. (2015) reported significant improvements in the psychological domains of the Menopause Rating Scale after 12 weeks of supplementation, suggesting broad symptom relief beyond vasomotor effects. Lipovac et al. (2012) also observed a notable 75.4% reduction in psychological symptoms during the active *T. pratense* phase of their crossover trial, compared with only 6.7% improvement during placebo. Further support comes from a systematic review and meta-analysis of 21 RCTs by Shahmohammadi et al. (2019), which found that phytoestrogen supplementation, including *T. pratense*, significantly reduced anxiety (SMD = -1.19 , $p = 0.04$) and depression (SMD = -0.95 , $p = 0.023$) compared with placebo. However, high heterogeneity ($I^2 > 90\%$), partly explained by differences in dosage, formulations, and study designs, limits the certainty of these findings. Notably, stronger effects were observed at doses of 80 mg/day or higher, consistent with the Lipovac et al. (2010) trial. These findings indicate that isoflavone-mediated modulation of oestrogen receptors may contribute to improved mood and emotional regulation. Although psychological outcomes are not always primary endpoints and measurement tools vary across studies, the overall pattern supports a beneficial effect of *T. pratense*, particularly with standardized doses around 80 mg/day. Improvements may be partially mediated through reductions in vasomotor symptoms, which are known to disrupt sleep, emotional stability, and overall quality of life (Lipovac et al., 2012). Overall, evidence supports a moderate improvement in psychological symptoms, with the strongest effects observed in studies using standardized isoflavone preparations.

Bone health

Oestrogen deficiency during menopause accelerates bone loss, and phytoestrogens have been investigated for their potential bone-protective effects (Thorup et al., 2015). Evidence specific to *T. pratense* remains limited but suggests some positive trends. Studies using approximately 37 mg/day of aglycone isoflavones have reported slowed bone turnover or modest improvements in bone-related biomarkers, although these results are not universally consistent (Thorup et al., 2015). Research in this area is constrained by small sample sizes, varied supplementation periods, and reliance on surrogate markers rather than clinically significant endpoints such as fracture reduction (Thorup et al., 2015). No large, long-duration RCTs have evaluated changes in bone mineral density over multiple years, which limits firm conclusions. Nonetheless, preliminary observations align with the known estrogenic activity of *T. pratense* isoflavones and suggest the potential for mild osteoprotective effects (Thorup et al., 2015). Overall, current evidence for bone-health benefits is suggestive but inconclusive, highlighting the need for larger, long-term trials assessing clinically relevant outcomes.

Safety profile

Overview

T. pratense has been mainly studied over 8-week to 12-month periods, with long-term use defined as more than 3 years, although data beyond this duration are limited. It is widely marketed for menopausal symptoms due to its isoflavone-mediated selective oestrogen receptor modulating (SERM) activity, which varies by manufacturing process (Papadopoulos et al., 2006). Across multiple clinical trials using standardized doses (40 to 160 mg/day isoflavones), *T. pratense* demonstrates favorable tolerability, with occasional rare adverse events and potential contamination-related risks requiring consideration (Atkinson et al., 2004; Lipovac et al., 2015; Taku et al., 2012).

General short- and medium-term safety

Randomized controlled trials consistently show good tolerability in peri- and postmenopausal women. In a 12-week double-blind RCT, 150 mL/day of extract (37.1 mg isoflavones) caused no reported side effects (Lipovac et al., 2015). No significant changes occurred in blood pressure or inflammatory cytokines, including IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, and IFN- γ (all $p > 0.05$). Markers of low-grade systemic inflammation also remained unchanged, indicating that *T. pratense* does not trigger inflammatory activation (Thorup et al., 2015). Cardiovascular parameters (systolic and diastolic blood

pressure) likewise showed no significant changes. Medium-term evidence further supports safety. In a 12-month trial, *T. pratense*-derived isoflavones (predominantly daidzein and glycitin) produced significant improvements in lipid profiles (total cholesterol, LDL, HDL) with no detectable adverse effects (Terzic et al., 2009). Clinical laboratory tests, physical examinations, and subjective assessments showed no toxicity. There were no baseline BMI differences ($p = 0.881$), and no safety issues emerged at monthly checkpoints. The absence of adverse-related dropouts in the intervention group further supports acceptability. Although limited by a small sample size and lack of blinding, the study suggests good long-term tolerability. Thorup et al. (2015) likewise confirmed the safety of 37.1 mg/day aglycone isoflavones over 12 weeks. No severe adverse events occurred, and adherence was 100%. No significant changes were observed in 24-h blood pressure. Lifestyle and dietary habits were controlled, and high baseline isoflavone consumers were excluded to reduce confounding.

Estrogenic effects and their implications

Mechanism of action

T. pratense isoflavones act as phytoestrogens with selective affinity for ER β over ER α (Papadopoulos et al., 2006; Ghazanfarpour et al., 2015; Karimpour-Reihan et al., 2018). ER β activation is associated with tumour-suppressive signaling, whereas ER α is linked to proliferation (Karimpour-Reihan et al., 2018). Isoflavones behave as partial agonists/antagonists, producing modest oestrogenic effects in tissues such as the vaginal epithelium while avoiding uterine or breast proliferation characteristic of pharmaceutical oestrogens (Atkinson et al., 2004). This receptor profile contributes to their favorable endometrial and breast safety (Ghazanfarpour et al., 2015).

Endometrial changes

Randomized controlled trials and meta-analyses consistently show that *T. pratense* does not increase endometrial thickness (Ghazanfarpour et al., 2015; Hale et al., 2001). Trials using 50 to 85.5 mg/day for 3 to 6 months found no significant differences versus placebo (Ghazanfarpour et al., 2015; Hale et al., 2001). Ghazanfarpour et al. (2015) reported a standardized mean difference (SMD) of 0.022 ($p = 0.915$; $I^2 = 0\%$), with no significant changes at 3, 6, 12, or 24 months. An exception is MF11RCE (80 mg/day), which reduced endometrial thickness over 12 weeks (-0.55 mm vs -0.18 mm; $p = 0.001$) without altering serum oestradiol ($p > 0.05$) (Imhof et al., 2006). MF11RCE appears safe for

postmenopausal women, although data in perimenopausal women are limited and it is likely unsuitable for premenopausal use. A pooled analysis of 2,167 women found that doses ≥ 54 mg/day produced a modest reduction (SMD -0.26 ; $p = 0.007$), particularly in North American cohorts (-0.23 mm; $p = 0.04$). Lower doses showed no effect (SMD 0.15 ; $p = 0.21$). Breakthrough bleeding was rare and comparable to placebo (Hale et al., 2001; Imhof et al., 2006).

Overall, evidence indicates no increase in endometrial thickness or hyperplasia for up to two years, with possible protective effects at higher standardized doses.

Breast tissue density and cancer risk

Given concerns with combined HRT (Powles et al., 2008), estrogenic safety is critical. Two RCTs using a *T. pratense* product standardized to 40 mg/day for 1 to 3 years in women at elevated breast cancer risk found no estrogenic effects (Atkinson et al., 2004; Powles et al., 2008; Fritz et al., 2013). No significant changes occurred in oestradiol, FSH, or LH ($p = 0.49$, $p = 0.84$, $p = 0.71$). Mammographic density remained stable. An ESR1 genotype interaction ($p = 0.009$) was noted: CC and CT genotypes showed small density reductions ($-3.4 \pm 9.7\%$ and $-5.2 \pm 12.0\%$), whereas TT genotypes showed a $1.4 \pm 12.3\%$ increase (Atkinson et al., 2004; Fritz et al., 2013); the clinical significance remains unclear. Powles et al. (2008) observed no differences in endometrial thickness, bone density, or mammographic measures in 401 high-risk women. Mechanistic data indicate that biochanin A and formononetin metabolize to genistein and daidzein, which preferentially activate ER β , contributing to anti-proliferative effects in breast tissue (Setchell and Clerici, 2010).

Safety in pregnancy and lactation

Due to their estrogenic activity, medicinal doses of *T. pratense* are not recommended during pregnancy or lactation (Foster, 2022; Association for the Advancement of Restorative Medicine, 2025). Although dietary exposure is common, pharmacologic doses could alter hormonal balance. Evidence is lacking, and potential risks remain theoretical; therefore, avoidance is advised.

Limitations and gaps in long-term safety data (>3 years)

Although *T. pratense* extracts demonstrate a favorable short- and medium-term safety profile, long-term (>3 years) safety data remain limited, particularly for oestrogen-sensitive tissues and coagulation-related outcomes. Most clinical trials assessing breast safety,

including mammographic density and circulating sex hormones, are restricted to 12–36 months, which is insufficient to detect slow-developing tissue changes or cancer-related endpoints (Atkinson et al., 2004; Powles et al., 2008; Fritz et al., 2013). Mammographic density is a surrogate marker, and existing studies do not evaluate histological changes or long-term breast cancer incidence (Powles et al., 2008). Inter-individual variability has been reported, including a significant interaction between ESR1 genotype and mammographic density, but trials are underpowered for genetic analyses and include predominantly Caucasian cohorts, limiting generalizability to diverse populations (Atkinson et al., 2004; Fritz et al., 2013). Women with prior breast cancer, BRCA mutations, or prolonged hormone therapy are routinely excluded, constraining assessment in high-risk groups (Powles et al., 2008). Long-term coagulation safety also remains insufficiently characterized. Isoflavones and related phenolic compounds in *T. pratense* exhibit antiplatelet activity in experimental models (Setchell and Clerici, 2010); however, clinical trials rely on standard coagulation tests (PT, aPTT, INR) that do not assess platelet function. A reported case of spontaneous subdural hematoma after 8 to 10 years of continuous *T. pratense* use, with normal routine coagulation but impaired platelet function, highlights this limitation (Hall et al., 2018). Although rare, this finding underscores the absence of platelet-specific monitoring and the lack of long-term safety data in older adults, individuals with comorbidities, and those receiving antiplatelet or anticoagulant therapy, who are systematically excluded from trials (Hall et al., 2018).

Potential drug interactions

CYP enzymes

Given *T. pratense*'s oestrogenic activity and its common concurrent use with conventional pharmacotherapies, particularly among peri- and postmenopausal women, concerns have emerged regarding its potential to alter drug metabolism via the cytochrome P450 (CYP) enzyme system (Chen et al., 2020). In vitro assays indicate that *T. pratense* constituents can inhibit CYP1A1/1A2, CYP1B1, CYP2C9, CYP2C8, CYP2C19, CYP2D6, and CYP3A4, among others (Arora et al., 2015; Kopečná-Zapletalová et al., 2017; Roberts et al., 2004; Sato et al., 2015; Wang et al., 2008). A study by Chen et al. (2020) assessed whether standardized *T. pratense* extract influenced the activity of key hepatic CYP enzymes, namely CYP1A2, CYP2C9, CYP2D6, and CYP3A4, in healthy peri- and postmenopausal women. The study employed validated probe substrates to evaluate pharmacokinetic parameters before and after *T. pratense* supplementation (Chen et al., 2020). Results showed no statistically or clinically significant changes in the pharmacokinetics of these

probe drugs (Chen et al., 2020). Although endogenous oestrogens are primarily metabolized by CYP3A4 (Zhang et al., 2024), most clinical studies of *T. pratense* have not systematically assessed routine liver enzyme parameters (e.g., Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP)) or potential interactions with CYP inducers or inhibitors, representing a limitation of the current evidence base (Chen et al., 2020).

Anticoagulant and antiplatelet considerations

Evidence from human trials shows that *T. pratense* does not exert clinically meaningful anticoagulant or antiplatelet effects at standard supplemental doses (Nestel et al., 1999; Teede et al., 2003; Wickham et al., 2022). Randomized controlled trials in postmenopausal women have noted improvements in vascular endpoints, including arterial stiffness and reduced adhesion molecules, but these reflect vascular modulation rather than direct effects on coagulation (Nestel et al., 1999; Teede et al., 2003; Wickham et al., 2022). Coagulation outcomes have been specifically tested in clinical trials. A randomized study of 150 postmenopausal women receiving 60.8 mg/day *T. pratense* isoflavones plus 19.2 mg/day soy isoflavones (total 80 mg/day) for six months found no significant changes in PT, aPTT, fibrinogen, or antithrombin III ($p > 0.05$) (Mainini et al., 2013). Thorup et al. (2015) similarly administered 150 mL/day of *T. pratense* extract (~37.1 mg isoflavones) for 12 weeks in 60 healthy menopausal women, with no significant effects on blood pressure, inflammatory biomarkers, or bleeding-related events. Additional trials using standardized isoflavone doses confirm these findings. Nestel et al. (1999) administered 40 mg/day and 80 mg/day sequentially in a crossover trial of 17 postmenopausal women, observing improved arterial compliance without changes in lipid profiles. Teede et al. (2003) replicated this in 18 women using 80 mg/day for five weeks, reporting reduced adhesion molecule levels but no coagulation effects. Wickham et al. (2022) administered 50 mL/day of fermented *T. pratense* extract (~37 mg/day isoflavones) for 14 days, reducing inflammatory markers such as VCAM-1 without altering haemostatic parameters.

Although clinical trials show no intrinsic anticoagulant activity, isolated case reports highlight the need for caution. Overall, the risk of interactions with warfarin appears low, though variability in supplement quality warrants prudence.

In summary, trials using standardized *T. pratense* isoflavone doses (~37–80 mg/day) consistently show no significant effects on PT, aPTT, fibrinogen, antithrombin III, or bleeding events. Reported coagulopathy cases likely stem from contaminated products rather than *T. pratense* itself. However, theoretical concerns involving

coumarins, possible CYP450 modulation, and formulation variability justify careful monitoring when *T. pratense* is used concomitantly with anticoagulant or antiplatelet medications.

Quality evaluation

Identity and authentication

Accurate botanical identification is essential to ensure that clinical outcomes reflect *T. pratense* rather than misidentified or adulterated plant material. In research settings, identification is performed by a trained taxonomist and verified through a deposited voucher specimen to ensure reproducibility (Booth et al., 2006a). After authentication, extracts undergo chemical profiling to confirm the presence of expected constituents—primarily daidzein, genistein, formononetin, and biochanin A and to verify the absence of harmful substances (Booth et al., 2006b). The phase II extract described by Booth et al. contained 35.54% total isoflavones, with coumarins and tyramine kept at $\leq 0.03\%$, minimizing anticoagulant and hypertensive risks. Its standardized clinical regimen of 397 mg/day delivered approximately 120 mg aglycone-equivalent isoflavones (Booth et al., 2006a). This level of authentication and chemical characterization ensured stability, predictable pharmacology, and GMP compliance (van Breemen, 2015). Because variations in isoflavone content significantly influence clinical outcomes, standardization is critical to ensure reproducibility (Wang et al., 2008; Andres et al., 2015).

Standardization challenges in *T. pratense* isoflavone preparations

Commercial *T. pratense* products vary widely in chemical composition compared with clinical-grade standardized extracts. In one evaluation of five marketed supplements, formononetin content varied 385-fold and biochanin A varied 45-fold; products also exhibited greater chromatographic impurities and altered intestinal transport characteristics (Wang et al., 2008). A German survey of 11 *T. pratense* and soy supplements showed intra-product variability up to 22%, with actual isoflavone contents ranging from 87 to 107% of label claims (Andres et al., 2015). Differences in extraction methods further contribute to inconsistent outcomes. Aqueous preparations contain higher levels of glycosides, whereas ethanolic and fermented extracts are enriched in aglycones such as biochanin A and formononetin. The isoflavone profile also shifts during maceration, resulting in additional variability (Malca-Garcia et al., 2019). These compositional differences likely influence efficacy: for example, standardized tablets such as Promensil® (40 mg/day) showed no estrogenic effects in high-risk women

(Atkinson et al., 2004), while aglycone-rich or fermented preparations demonstrated reductions in vasomotor symptoms and inflammatory markers (Lambert et al., 2017; Wickham et al., 2022), although benefits are not uniform across all symptom domains (Villadsen et al., 2023). To address this inconsistency, Health Canada's 2025 Red Clover Isoflavone Extract monograph mandates potency reporting as Aglycone Isoflavone Equivalents (AIE), with recommended intakes of 40–100 mg AIE for menopausal use (Health Canada, 2025).

Standardization and regulatory guidance

Robust quality control requires analytical methods capable of verifying both chemical identity and quantitative composition. The UIC/NIH Botanical Research Centre developed a clinical-grade isoflavone-enriched extract analyzed using UHPLC–UV alongside quantitative ^1H NMR (qHNMR), enabling simultaneous monitoring of 11 diagnostic compounds (nine isoflavones and two flavanols) (Phansalkar et al., 2017). qHNMR results were consistent with chromatographic data, and the extract remained chemically stable for nearly a decade. These findings highlight the value of multimarker qHNMR for improving analytical accuracy, establishing mass balance, and distinguishing authentic extracts from adulterated materials (Phansalkar et al., 2017). International regulatory approaches differ considerably. Health Canada requires AIE-based potency reporting and recommends 40 to 100 mg/day for menopausal indications (Health Canada, 2025). In contrast, the European Medicines Agency permits simplified registration for traditional herbal products with ≥ 30 years of documented use but does not require potency reporting (European Parliament and Council [EPC], 2004; European Medicines Agency [EMA], 2022). The U.S. FDA regulates red clover under DSHEA, which mandates GMP compliance but does not require pre-market potency testing or standardized isoflavone labeling (U.S. Congress, 1994; FDA, 2015). South African Health Products Regulatory Authority (SAHPRA) categorizes *T. pratense* under Category D “Red Clover isoflavone extract,” allowing multiple plant parts and requiring GMP compliance, AIE potency reporting, and standard contraindications such as pregnancy, breastfeeding, and concurrent use with estrogens or anticoagulants, including warfarin (SAHPRA, 2022).

Compared with the EMA and FDA, which do not mandate potency reporting, the AIE-based frameworks of Health Canada and SAHPRA better address known variability in isoflavone content (Andres et al., 2015; Wang et al., 2008).

To ensure reproducibility of the ~80 mg/day efficacy observed in vasomotor symptom trials, standardization to total isoflavones expressed as aglycone isoflavone equivalents is recommended, as isoflavones are

consumed mainly as glycosides while biological activity is mediated by aglycones acting preferentially at ER β , and glycoside-to-aglycone conversion varies with gut β -glucosidase activity and microbiota composition (Papadopoulos et al., 2006; Setchell and Clerici, 2010). Harmonized adoption of AIE labeling would therefore improve analytical consistency, clinical reliability, and cross-study comparability (van Breemen, 2015).

Contaminants and adulteration

Heavy metal contamination is a persistent quality issue across herbal supplements, including *T. pratense*. A global analysis of 1,773 herbal medicines reported that 30.51% exceeded pharmacopoeial limits for at least one heavy metal cadmium, lead, arsenic, mercury, or copper using ICP-MS (Luo et al., 2020). Similarly, a systematic review found cadmium and lead in 68% of supplements tested and mercury in 29%, demonstrating widespread contamination across international markets (Ćwieląg-Drabek et al., 2020). These metals pose risks of organ toxicity and carcinogenesis, emphasizing the need for routine contaminant screening. Adulteration also presents risks. One case report described a 28-year-old woman who developed a bleeding disorder after consuming a herbal tea containing *T. pratense* and alfalfa; analysis revealed warfarin contamination despite no prescription use (Karimpour-Reihan et al., 2018). Such cases indicate that adverse events may arise from contamination rather than the intrinsic effects of *T. pratense*, underscoring the importance of stringent quality assurance.

Conclusion

Across more than 30 human clinical studies, evidence indicates that standardized and analytically verified *T. pratense* extracts provide reproducible isoflavone exposure, modest symptom benefits, and a favorable safety profile. However, commercial supplements show substantial variability in isoflavone content, elemental impurities, and pharmacokinetic behavior, limiting the consistency of clinical outcomes (Booth et al., 2006b; Andres et al., 2015; Luo et al., 2020). Efficacy appears formulation-dependent. Standardized tablets generally show no effects on mammographic density or gonadotropins (Atkinson et al., 2004), whereas fermented or aglycone-enriched extracts have demonstrated reductions in vasomotor symptoms and inflammatory markers such as VCAM-1 (Lambert et al., 2017; Wickham et al., 2022).

Benefits are most consistent for vasomotor symptoms, with improvements typically observed at ≥ 80 mg/day of isoflavones. Evidence also supports improvements in vaginal dryness and atrophy, including from topical formulations, while psychological outcomes (anxiety, low mood) show moderate but less consistent improvements

Limited studies suggest potential preservation of bone mineral density at ~ 37 mg/day aglycone isoflavones. Breast tissue safety is well supported, with no increases in density or circulating oestradiol and some genotype-dependent variability in response (Atkinson et al., 2004; Fritz et al., 2013). Safety concerns arise primarily from contamination and adulteration rather than intrinsic pharmacology. Heavy-metal excess occurs in up to 30% of global herbal supplements (Luo et al., 2020), and isolated bleeding events have been linked to adulterated products (Karimpour-Reihan et al., 2018). Controlled trials show no significant effects on coagulation parameters or CYP metabolism, supporting a low risk of pharmacokinetic interactions, though caution remains appropriate with anticoagulants, HRT, and SERMs.

Regulatory frameworks remain inconsistent. Health Canada mandates potency reporting using Aglycone Isoflavone Equivalents (40 to 100 mg/day), while the EMA and FDA do not, hindering dose comparability across studies. Universal AIE labeling and routine contaminant screening would improve clinical reliability. Overall, standardized *T. pratense* extracts represent a safe and potentially beneficial adjunctive therapy for menopausal symptoms, particularly vasomotor and urogenital complaints, when used at evidence-based doses. Remaining gaps include short trial durations, heterogeneous formulations, limited mechanistic data, and underrepresentation of diverse populations. Future research should prioritize long-term, formulation-specific trials, harmonized analytical standards, and dedicated herb–drug interaction studies to strengthen the evidence base.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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