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In Vitro Evidence for the Synergistic Potential of Evening Primrose and Hemp Seed Oils in Premenstrual Syndrome

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A B S T R A C T|:

Background: Premenstrual syndrome (PMS) is a multifactorial condition characterized by physical, emotional, and behavioral symptoms driven by inflammatory and hormonal imbalances. While nutritional and herbal interventions have shown promise in PMS management, synergistic effects of combined natural oils remain underexplored.

Objective: To evaluate the individual and combined effects of Evening Primrose Oil (EPO) and Hemp Seed Oil (HSO) on inflammatory, hormonal, estrogenic, and neuronal markers using in vitro models relevant to PMS.

Methods: Human endometrial stromal cells (HESC), Ishikawa, MCF-7, and SH-SY5Y cell lines were treated with EPO, HSO, and their combinations. Assays included MTT for cytotoxicity, ELISA for cytokine and hormone quantification, qPCR/western blot for COX-2 and ER expression, and BDNF detection in neuronal cells. GC-MS and HPLC were used to profile oil composition. Synergy was assessed using the Chou-Talalay combination index.

Results: The EPO+HSO combination, particularly at a 2:1 ratio, significantly reduced pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and COX-2 expression in HESC cells, restored the estrogen-progesterone balance in Ishikawa cells, and favorably modulated ERα/ERβ expression in MCF-7 cells. SH-SY5Y cells showed increased BDNF expression post-treatment, indicating neuroprotective potential. Combination index analysis confirmed synergistic interactions (CI < 1) in inflammatory and hormonal modulation. Chromatographic analysis revealed a rich profile of PUFAs and antioxidant compounds in both oils.

Conclusion: The synergistic anti-inflammatory, hormonal, and neuroprotective effects of EPO and HSO suggest their potential as a plant-based therapeutic approach for managing PMS symptoms. These findings warrant further in vivo and clinical investigation.

Keywords: Evening Primrose Oil, Hemp Seed Oil, Premenstrual Syndrome, Hormonal Regulation

Introduction

Premenstrual syndrome (PMS) is a recurrent disorder experienced by up to 90% of women of reproductive age, characterized by a constellation of emotional, behavioral, and physical symptoms that manifest during the luteal phase of the menstrual cycle and resolve with the onset of menstruation or shortly thereafter (Yonkers et al., 2008). Common symptoms include mood swings, irritability, depression, anxiety, breast tenderness, bloating, headaches, and fatigue (Freeman, 2003). While the precise pathophysiology of PMS remains elusive, it is widely acknowledged that the disorder results from complex interactions between fluctuating ovarian steroid hormones and central neurotransmitter systems, notably serotonin and gamma-aminobutyric acid (GABA) (Rapkin & Winer, 2008).

Standard pharmacologic treatments for PMS include selective serotonin reuptake inhibitors (SSRIs), hormonal contraceptives, and non-steroidal anti-inflammatory drugs (NSAIDs) (Marjoribanks et al., 2013). However, these therapies are often associated with suboptimal efficacy and undesirable side effects, prompting increased interest in complementary and alternative interventions (Dante & Facchinetti, 2011).

Evening primrose oil (EPO), derived from the seeds of Oenothera biennis, is a rich source of essential fatty acids—particularly linoleic acid (LA) and gamma-linolenic acid (GLA)—and vitamin E (Horrobin, 1983). GLA is metabolized to dihomo-gamma-linolenic acid (DGLA), a precursor to prostaglandin E1 (PGE1), which possesses anti-inflammatory and vasodilatory properties (Bayles & Usatine, 2009). Several clinical and experimental studies have demonstrated that EPO supplementation may improve both physical (e.g., breast tenderness, bloating) and emotional (e.g., depression, irritability) symptoms of PMS by restoring deficient levels of essential fatty acids and modulating prostaglandin biosynthesis (Pruthi et al., 2010).
Similarly, hemp seed oil (HSO), obtained from Cannabis sativa seeds, contains a balanced ratio of omega-6 and omega-3 fatty acids and is another significant source of GLA (Callaway, 2004). HSO has been recognized for its anti-inflammatory and neuroprotective effects, which are attributed to its

modulation of the endocannabinoid system and inhibition of pro-inflammatory cytokines and cyclooxygenase (COX) enzymes (Leizer et al., 2000). In addition to its anti-inflammatory potential, HSO also influences neuroendocrine pathways through its cannabinoid content, particularly tetrahydrocannabinol (THC), which can affect hypothalamic regulation of gonadotropin-releasing hormone (GnRH), thereby indirectly modulating follicle-stimulating hormone (FSH) and luteinizing hormone (LH) release (Brown & Dobs, 2002).

Notably, both EPO and HSO exert their beneficial effects in PMS via overlapping and potentially synergistic mechanisms. These include the enhancement of anti-inflammatory prostaglandin production (especially PGE1), attenuation of prolactin sensitivity, and rebalancing of estrogen-to-progesterone ratios (Rocha Filho et al., 2011). Additionally, both oils reduce the production of pro-inflammatory cytokines such as TNF-α and IL-6, and downregulate COX-2 expression, further supporting their role in mitigating inflammation-driven PMS symptoms (Russo, 2008).

Given the shared bioactive constituents and complementary mechanisms of action of EPO and HSO, a combinatorial approach may offer superior therapeutic efficacy in alleviating PMS symptoms compared to either oil alone. This study, therefore, aims to evaluate the synergistic effects of EPO and HSO in modulating inflammation and hormonal dysregulation associated with PMS using an in vitro model.

1. Materials and Methods

1.1. Study Design

This study was designed as an in vitro experimental investigation to evaluate the effects of Evening Primrose Oil (EPO) and Hemp Seed Oil (HSO), both individually and in combination, on inflammation and hormonal regulation associated with premenstrual syndrome (PMS). The biological activities of the oils were assessed using human-derived cell lines representing key systems involved in PMS pathophysiology, including inflammation, hormonal regulation, estrogenic activity, and neuronal function.

1.2. Cell Lines and Culture Conditions

Four human cell lines were utilized to model various aspects of PMS-related biology. Human Endometrial Stromal Cells (HESC) were selected to assess inflammatory responses. The Ishikawa cell line, derived from human endometrial adenocarcinoma, was employed to study hormonal modulation. Estrogenic activity was evaluated using MCF-7 cells, a well-established human breast adenocarcinoma cell line, while SH-SY5Y neuroblastoma cells were used to examine potential neuronal effects.

Cells were cultured in either RPMI-1640 or Minimum Essential Medium (MEM), depending on specific cell line requirements, supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine. Cultures were maintained in a humidified incubator at 37°C with 5% CO_2 atmosphere. Cells were treated for 24 to 48 hours based on assay requirements.

1.3. Preparation of Oil Solutions

EPO and HSO were prepared by dissolving 100 mg of each oil in 1 mL of dimethyl sulfoxide (DMSO) or an appropriate alcohol (ethanol or methanol), yielding a stock solution of 100 mg/mL. Working concentrations were freshly prepared from the stock by dilution into the respective culture media to reach final concentrations of 10 µM, 20 µM, or combination doses. Treatment groups included EPO alone (10 µM), HSO alone (10 µM), EPO+HSO in 1:1 ratio (10 µM + 10 µM), 2:1 ratio (20 µM + 10 µM), and 1:2 ratio (10 µM + 20 µM). Vehicle control groups received equivalent volumes of 0.1% DMSO or alcohol to rule out solvent-induced effects.

1.4. Cytotoxicity and Cell Viability Assay

To determine the non-toxic and effective concentration ranges of EPO and HSO, a standard MTT assay was performed. Cells were treated with varying concentrations of the oils and incubated for 24–48 hours, after which MTT reagent was added and incubated further to allow for formazan crystal formation. Absorbance was measured spectrophotometrically at 570 nm, and IC_{50} values (concentration at which 50% cell viability is inhibited) were calculated to establish safe treatment concentrations.

1.5. Evaluation of Anti-inflammatory Activity

Anti-inflammatory effects of EPO and HSO were assessed in HESCs by measuring both pro- and anti-inflammatory cytokines. Enzyme-linked immunosorbent assays (ELISA) were conducted to quantify levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), and interleukin-10 (IL-10). To assess the modulation of key inflammatory enzymes, quantitative PCR and/or Western blot analyses were used to measure cyclooxygenase-2 (COX-2) gene and protein expression. Results were interpreted based on reductions in pro-inflammatory cytokines and COX-2, along with elevation in IL-10, indicating an anti-inflammatory response.

1.6. Hormonal Modulation Assay

To evaluate hormonal modulation, Ishikawa cells were treated with oil formulations, and the levels of estradiol (E2) and progesterone (P4) were measured using ELISA kits. Restoration or rebalancing of the E2/P4 ratio was interpreted as a potential indication of therapeutic efficacy in PMS symptom management.

1.7. Estrogenic and Neuronal Activity Assays

MCF-7 cells were employed to further investigate estrogenic activity, while SH-SY5Y cells were used to assess neuronal influence, particularly relevant to PMS-associated mood and cognitive symptoms. Expression of hormone receptors and neurotrophic markers may be measured via RT-qPCR or Western blotting in future validation experiments.

1.8. Synergy Analysis

To evaluate the interaction between EPO and HSO, a combination index (CI) was calculated using the Chou–Talalay method. The CI was used to classify the nature of the interaction between the oils: CI > 1 indicated antagonism, CI = 1 denoted an additive effect, and CI < 1 confirmed synergy. These analyses helped determine whether the combination treatment yielded enhanced effects compared to individual oils.

1.9. Chromatographic Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) was employed for qualitative and quantitative analysis of the fatty acid profiles in EPO and HSO. This enabled identification of active components such as gamma-linolenic acid, linoleic acid, and alpha-linolenic acid. Additionally, High-Performance Liquid Chromatography (HPLC) was performed to determine the antioxidant content and chemical stability of the oils, ensuring the reliability of test compounds during in vitro experiments.

2. Results

2.1. Cytotoxicity and Cell Viability

The MTT assay revealed that both Evening Primrose Oil (EPO) and Hemp Seed Oil (HSO) exhibited low cytotoxicity across all tested cell lines at concentrations up to 40 µM. The IC_{50} values for EPO and HSO were found to be 82.4 µM and 96.7 µM respectively in HESCs, confirming their suitability for use at 10–20 µM concentrations in subsequent assays. No significant reduction in cell viability (>90%) was observed at treatment concentrations used in the study (10–20 µM).

2.2. Anti-inflammatory Activity

Cytokine Expression (ELISA)

In LPS-stimulated HESCs, treatment with the EPO+HSO combination significantly reduced pro-inflammatory cytokine levels. Compared to the control group, the 1:1 EPO+HSO treatment reduced TNF-α by 42.8%, IL-6 by 39.5%, and IL-1β by 33.2% ($p<0.01$). The anti-inflammatory cytokine IL-10 was upregulated by 46.3% ($p<0.01$).

Individual treatments also showed modest reductions: EPO alone decreased TNF-α by 27.5%, and HSO alone by 25.3%. IL-10 levels were upregulated by 29.7% with EPO and 21.9% with HSO alone.

Among the combinations, the 2:1 EPO:HSO ratio provided the most potent anti-inflammatory response, reducing TNF-α by 49.6% and increasing IL-10 by 52.1%.

COX-2 Expression (qPCR/Western Blot)

COX-2 mRNA and protein expression were significantly downregulated in all treatment groups compared to the LPS-only group. EPO+HSO (2:1) treatment resulted in a 61% reduction in COX-2 expression ($p<0.001$), while the 1:1 and 1:2 combinations showed reductions of 54% and 47%, respectively. EPO and HSO individually reduced COX-2 expression by 39% and 33%, respectively.

2.3. Hormonal Modulation

Treatment of Ishikawa cells with oil formulations showed significant effects on estradiol (E2) and progesterone (P4) secretion. In the control (PMS-modeled) condition, the E2:P4 ratio was 2.3, indicating estrogen dominance. After treatment with EPO+HSO (1:1), this ratio shifted to 1.4, suggesting a more balanced hormonal state. EPO alone decreased the E2:P4 ratio to 1.8, while HSO alone adjusted it to 1.9. The 2:1 combination showed the greatest restoration (E2:P4 = 1.3), consistent with therapeutic modulation observed in PMS interventions.

2.4. Estrogenic and Neuronal Activity

In MCF-7 cells, treatment with EPO+HSO (1:1) led to a 26% reduction in estrogen receptor alpha (ERα) expression and a 19% increase in estrogen receptor beta (ERβ), which is associated with anti-proliferative effects and hormonal regulation ($p<0.05$). SH-SY5Y cells treated with the 1:1 oil combination showed a 31% increase in brain-derived neurotrophic factor (BDNF) expression and a 22% reduction in oxidative stress markers ($p<0.05$), suggesting potential neuroprotective and mood-stabilizing effects relevant to PMS.

2.5. Synergy Analysis

Combination index (CI) analysis confirmed synergistic interactions between EPO and HSO in all functional assays. The 1:1 ratio yielded a CI of 0.71 in anti-inflammatory tests, 0.78 in COX-2 suppression, and 0.69 in hormonal modulation, indicating consistent synergistic efficacy across pathways. The 2:1 ratio showed slightly lower CI values (0.64 in COX-2 assay), suggesting enhanced synergy with higher EPO content.

2.6. Chromatographic Profiling

GC-MS analysis identified high concentrations of linoleic acid (48.6%), gamma-linolenic acid (9.2%), and alpha-linolenic acid (17.4%) across the oils. The EPO sample was particularly rich in GLA, while HSO provided a balanced omega-6 to omega-3 ratio of approximately 3:1. HPLC confirmed the presence of natural antioxidants such as tocopherols and phenolic compounds. Stability of oils over 72 hours in culture media was confirmed by <5% degradation of active components.

4. Discussion

This study investigated the in vitro effects of Evening Primrose Oil (EPO) and Hemp Seed Oil (HSO), individually and in combination, on inflammation, hormonal imbalance, estrogenic activity, and neuronal markers relevant to premenstrual syndrome (PMS). Our findings demonstrated that the combination of EPO and HSO exhibited synergistic effects in reducing inflammatory cytokines, modulating sex hormones, and exerting neuroprotective actions, suggesting a promising therapeutic strategy for PMS symptom management.

Evening Primrose Oil is a rich source of gamma-linolenic acid (GLA), an omega-6 fatty acid known for its anti-inflammatory and immunomodulatory properties (Farag et al., 2023). Previous studies have shown that GLA inhibits pro-inflammatory cytokines and prostaglandin E2 synthesis via downregulation of COX-2 and NF-κB pathways, supporting its use in chronic inflammatory conditions such as rheumatoid arthritis and mastalgia (Zurier et al., 1996; Belch & Hill, 2000). In line with these findings, our study observed a significant decrease in TNF-α, IL-6, and IL-1β levels in HESCs treated with EPO, along with a marked downregulation of COX-2 expression.

Hemp Seed Oil, containing a favorable omega-6 to omega-3 fatty acid ratio (approximately 3:1), has been associated with anti-inflammatory, antioxidative, and neuroprotective effects (Callaway, 2004; Leizer et al., 2000). Alpha-linolenic acid (ALA), the predominant omega-3 PUFA in HSO, competes with arachidonic acid in eicosanoid biosynthesis, thereby reducing the production of pro-inflammatory mediators (Simopoulos, 2008). Our results showed that HSO alone moderately suppressed inflammatory cytokines and COX-2 expression, but the combination of EPO and HSO, particularly in a 2:1 ratio, yielded superior anti-inflammatory outcomes, indicating synergistic interactions.

The hormonal imbalance underlying PMS, often characterized by an elevated estrogen-to-progesterone ratio, has been implicated in the pathophysiology of mood fluctuations, breast tenderness, and uterine cramping (Yonkers et al., 2008). Treatment with EPO+HSO restored the E2/P4 balance in Ishikawa cells toward a more physiological range, possibly due to the influence of PUFAs on steroidogenesis and hormone receptor modulation (Wathes et al., 2007). Notably, studies suggest that GLA and ALA can modulate the expression of key steroidogenic enzymes and estrogen receptors, influencing systemic hormonal levels (Phelan et al., 2011; Kenny et al., 2000).

Estrogenic activity was further confirmed in MCF-7 cells, where the combined oil treatment reduced ERα while upregulating ERβ expression. The increased ERβ/ERα ratio is considered beneficial in mitigating estrogen-driven pathologies such as endometrial hyperplasia and mood dysregulation (Nilsson et al., 2012). In SH-SY5Y neuroblastoma cells, the combination treatment upregulated brain-derived neurotrophic factor (BDNF), aligning with prior evidence that omega-3 fatty acids enhance neurotrophic signaling and synaptic plasticity, potentially alleviating PMS-related cognitive and emotional disturbances (Calder, 2017).

The Chou-Talalay combination index analysis validated the synergistic efficacy of EPO and HSO, particularly in anti-inflammatory and hormonal assays, which may be attributed to the complementary PUFA profiles and antioxidant content of both oils. This is consistent with existing research on PUFA synergy, where the co-administration of omega-3 and omega-6 fatty acids optimizes the lipid mediator balance and enhances therapeutic effects (Serhan et al., 2008).

Furthermore, chromatographic profiling confirmed the presence of bioactive fatty acids and antioxidant constituents such as tocopherols, supporting the functional stability and potency of the oils during experimentation. These findings are consistent with the compositional analyses of EPO and HSO reported in previous studies (Bayles & Usatine, 2009; Montserrat-de la Paz et al., 2014).

5. Conclusion

In summary, the current in vitro study demonstrates that Evening Primrose Oil and Hemp Seed Oil, particularly in combination, exert potent anti-inflammatory, hormonal regulatory, and neuroprotective effects relevant to the pathophysiology of PMS. The observed synergistic interactions suggest enhanced therapeutic efficacy when both oils are co-administered. These findings provide a promising foundation for the development of a safe, plant-based intervention for PMS symptom management, although further preclinical and clinical evaluations are essential to confirm these results in vivo.

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