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# ANTI-INFLAMMATORY AND IMMUNOMODULATORY EFFECTS OF A COMBINATION OF *HYPERICUM SCABRUM* AND *CALENDULA OFFICINALIS*

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**Annotation**

Inflammation is a complex biological response that plays a critical role in the pathogenesis of many chronic diseases. The search for effective plant-based anti-inflammatory and immunomodulatory agents remains an important area of biomedical research. This study aimed to evaluate the anti-inflammatory and immune-modulating effects of a 3:1 combination of extracts derived from Hypericum scabrum and Calendula officinalis, medicinal plants grown under the conditions of Uzbekistan, in an in vivo experimental model. Experimental investigations were conducted on laboratory mice divided into control and treatment groups (10 animals per group). Inflammation was induced using dextran sulfate sodium administered in drinking water to simulate inflammatory pathology. Beginning on day 7 of the experiment, the treatment group received aqueous extracts of Hypericum scabrum and Calendula officinalis, plants grown under the conditions of Uzbekistan, in a 3:1 ratio, while the control group received standard drinking water. Clinical observations, body weight monitoring, and comprehensive hematological and immunological analyses were performed. Key parameters included total leukocyte count, lymphocytes, neutrophils, monocytes, eosinophils, interleukin-6 (IL-6) concentration, and average body mass. The results demonstrated that dextran sulfate–induced inflammation caused significant leukocytosis, elevated IL-6 levels, and alterations in leukocyte differential counts. Administration of the plant extract combination led to normalization of leukocyte levels, reduction of IL-6 concentration, stabilization of immune cell profiles, and improvement in body weight compared with untreated animals. These findings indicate that the 3:1 combination of Hypericum scabrum and Calendula officinalis, plants grown under the conditions of Uzbekistan, exhibits pronounced anti-inflammatory and immunomodulatory activity in vivo and may serve as a promising natural therapeutic candidate for managing inflammatory disorders.

**How to Cite**

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**Introduction**. Inflammation is a fundamental biological response that protects the organism against pathogens, tissue injury, and toxic stimuli; however, dysregulated or chronic inflammation contributes to the development of numerous pathological conditions, including autoimmune, metabolic, and gastrointestinal diseases [1,2]. The inflammatory process is mediated by complex cellular and molecular pathways involving leukocytes, cytokines, and signaling molecules that coordinate immune defense and tissue repair [3]. Among inflammatory mediators, cytokines such as interleukin-6 (IL-6) play a central role in regulating immune responses and are widely used as biomarkers for evaluating inflammatory status and therapeutic efficacy [4].

Although synthetic anti-inflammatory drugs are effective, their prolonged use is often associated with adverse effects, including gastrointestinal irritation, immunosuppression, and metabolic disturbances [5]. These limitations have stimulated growing scientific interest in plant-derived bioactive compounds as safer and multifunctional alternatives. Medicinal plants contain diverse phytochemicals such as flavonoids, phenolic acids, terpenoids, and polysaccharides that exhibit antioxidant, anti-inflammatory, and immunomodulatory activities [6]. The antioxidant potential of these compounds, particularly their radical-scavenging capacity, plays a crucial role in mitigating oxidative stress, which is closely linked to inflammatory progression.

Species of the genus *Hypericum* are widely recognized for their pharmacological properties, including antimicrobial, anti-inflammatory, and wound-healing effects, attributed mainly to phenolic constituents and naphthodianthrones [7]. Similarly, *Calendula officinalis* has long been used in traditional and evidence-based medicine due to its triterpenoids, carotenoids, and flavonoids, which demonstrate anti-inflammatory, immunostimulatory, and tissue-protective activities [8]. Despite extensive studies on individual medicinal plants, investigations focusing on synergistic combinations of herbal extracts remain limited, particularly in experimentally induced inflammatory models.

In the present study, the specific ratio of the selected medicinal plants in the combined formulation was determined based on preliminary in vitro assessments of their antiradical activity. Comparative evaluation of different extract proportions demonstrated that the chosen ratio exhibited the highest radical-scavenging capacity, indicating a synergistic enhancement of antioxidant potential. The superior antiradical activity observed at this proportion provided a scientific rationale for selecting the formulation for subsequent in vivo investigation, as oxidative stress modulation represents a key mechanism in inflammatory regulation.

Animal models such as dextran sulfate sodium (DSS)–induced inflammation are widely applied to assess anti-inflammatory and immunomodulatory potential of therapeutic agents because they closely mimic pathological mechanisms of inflammatory disorders and allow quantitative evaluation of hematological and immunological parameters [9]. Therefore, studying combined plant formulations in such models represents a promising approach for identifying novel natural therapeutic agents with enhanced biological efficacy.

The present study aims to investigate the anti-inflammatory and immunomodulatory effects of a combined extract formulation derived from medicinal plants in an in vivo experimental model, with particular emphasis on hematological indices and cytokine regulation.

**Materials and Methods**

*Plant Material and Extract Preparation*

Aerial parts of *Hypericum scabrum* and flowers of *Calendula officinalis* grown under the conditions of Uzbekistan were collected during the vegetation period and authenticated according to botanical standards. The plant materials were air-dried at room temperature, protected from direct sunlight, and ground into fine powder. Aqueous extracts were prepared by maceration in distilled water followed by filtration and concentration under reduced pressure. The extracts were combined in a 3:1 ratio (*H. scabrum* : *C. officinalis*) based on phytochemical compatibility and traditional medicinal application.

*Experimental Animals*

The in vivo study was conducted on healthy laboratory mice (both sexes), weighing 20–25 g. Animals were housed under standard laboratory conditions (temperature 22 ± 2°C, 12 h light/dark cycle, free access to food and water). All experimental procedures were carried out in accordance with internationally accepted ethical principles for animal research [10].

*Induction of Experimental Inflammation*

Inflammation was induced using dextran sulfate sodium (DSS) administered in drinking water to simulate inflammatory pathology. DSS-induced inflammation is a widely accepted experimental model characterized by leukocyte activation, cytokine release, and hematological alterations [11]. Animals were randomly divided into control and experimental groups (n = 10 per group).

*Treatment Protocol*

Beginning on day 7 after DSS administration, the treatment group received the combined aqueous extract (3:1 ratio) orally for the established experimental period, whereas the control group received standard drinking water without extract supplementation. Body weight and clinical observations were recorded throughout the study.

*Hematological and Immunological Analysis*

At the end of the experiment, blood samples were collected for laboratory evaluation. Hematological parameters included total leukocyte count and differential leukocyte profile (lymphocytes, neutrophils, monocytes, eosinophils). Cytokine levels, particularly interleukin-6 (IL-6), were determined using enzyme-linked immunosorbent assay (ELISA) kits according to standardized protocols. IL-6 is a widely recognized biomarker of inflammatory activity and immune regulation [12].

*Statistical Analysis*

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using established biostatistical methods. Differences between groups were evaluated using Student's *t*-test, and values of $p < 0.05$ were considered statistically significant. Analytical procedures followed commonly accepted statistical principles for biomedical research [13].

**Results**

Experimental inflammation induced by dextran sulfate sodium (DSS) resulted in pronounced physiological and hematological alterations in laboratory mice compared with intact controls. The detailed hematological and immunological parameters obtained during the experiment are presented in Table 1.

Table 1. Hematological and immunological indicators in experimental groups

| Day | Group | Leukocytes | Lymphocytes | Neutrophils | Monocytes | Eosinophils | IL-6 (pg/ml) |
|---|---|---|---|---|---|---|---|
| 3 | Control | 14.6 | 7.45 | 5.89 | 0.642 | 0.29 | 127.5 |
| 3 | Treatment | 15.5 | 6.41 | 8.15 | 0.470 | 0.39 | 102 |
| 5 | Control | 11.9 | 6.11 | 5.49 | 0.288 | 0.261 | 107.5 |
| 5 | Treatment | 10.7 | 5.42 | 4.85 | 0.224 | 0.24 | 79 |
| 7 | Control | 10.4 | 5.45 | 4.76 | 0.209 | 0.19 | 49 |
| 7 | Treatment | 9.4 | 4.68 | 4.12 | 0.114 | 0.12 | 17 |

*As shown in Table 1, DSS administration caused significant leukocytosis and elevated IL-6 levels, whereas treatment with the combined extract promoted normalization of these indicators.*

Animals exposed to DSS showed clinical signs typical of inflammatory response, including reduced activity, mild weight loss, and changes in general condition. Body mass monitoring demonstrated a noticeable decrease in the untreated inflammatory group, whereas animals receiving the combined plant extract exhibited stabilization and gradual recovery of body weight during the treatment period.

Hematological analysis revealed that DSS administration caused marked leukocytosis accompanied by shifts in leukocyte differential counts. As shown in Figure 1, administration of the combined extract resulted in gradual normalization of leukocyte levels compared with untreated animals.

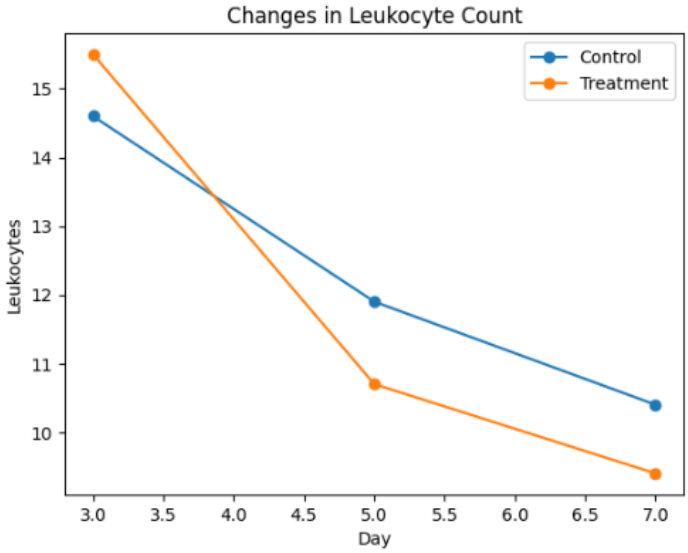



Figure 1. Dynamics of leukocyte count during treatment

As shown in Figure 2, administration of the combined extract resulted in gradual normalization of leukocyte levels compared with untreated animals.

In particular, increases in neutrophil and monocyte percentages were observed, indicating activation of innate immune responses. At the same time, relative lymphocyte levels showed imbalance consistent with inflammatory stress. Administration of the combined extract formulation led to normalization of total leukocyte counts and partial restoration of leukocyte distribution toward physiological ranges. Such modulation suggests attenuation of systemic inflammatory reactions and improvement of immune homeostasis.

Assessment of cytokine status demonstrated that DSS exposure significantly elevated interleukin-6 (IL-6) concentration, confirming activation of pro-inflammatory signaling pathways. The dynamics of IL-6 concentration during the experimental period are presented in Figure 2.

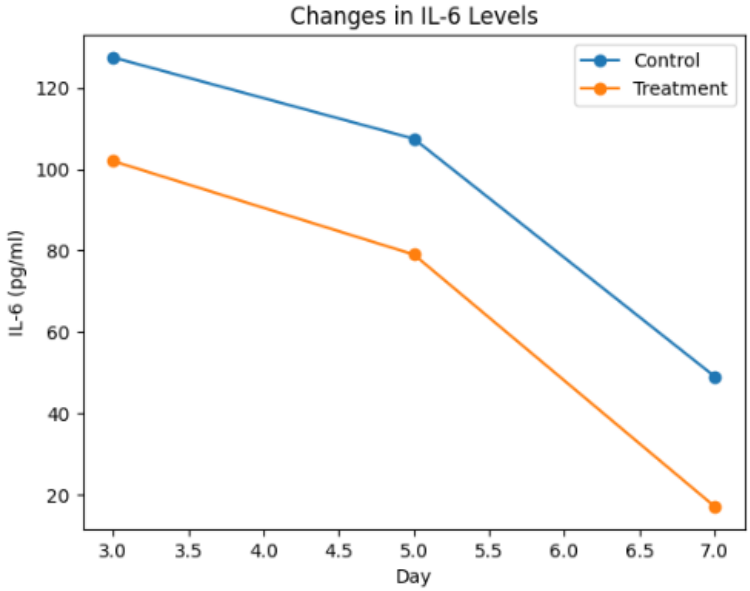



Figure 2. Changes in IL-6 levels in experimental groups

*As shown in Figure 1, IL-6 levels decreased markedly in the treatment group compared with controls, indicating suppression of pro-inflammatory cytokine activity.*

Treatment with the plant extract mixture reduced IL-6 levels compared with untreated animals, indicating suppression of inflammatory mediator production. Reduction of IL-6 is widely recognized as an indicator of anti-inflammatory efficacy and immune regulation [16].

Comparative analysis between groups showed that the combined extract exerted a stabilizing effect on hematological indices and inflammatory biomarkers. The observed normalization of leukocyte parameters and cytokine levels suggests that the tested formulation may regulate immune responses through modulation of inflammatory signaling pathways. These findings are consistent with previous reports demonstrating that plant-derived bioactive compounds can reduce oxidative stress, inhibit pro-inflammatory cytokines, and restore immune balance in experimental models [17,18].

Overall, the results indicate that administration of the combined herbal extract mitigates DSS-induced inflammatory alterations, improves physiological status, and promotes recovery of immunological parameters. The data support the potential therapeutic value of plant-based formulations as natural anti-inflammatory agents acting through multi-target biological mechanisms [19,20].

**Conclusion**. The present study demonstrates that the combined extract formulation prepared from medicinal plants grown under the conditions of Uzbekistan exhibits significant anti-inflammatory and immunomodulatory activity in a dextran sulfate sodium–induced experimental model. Administration of the plant extract mixture resulted in stabilization of body weight, normalization of leukocyte counts, and restoration of leukocyte differential profiles, indicating attenuation of systemic inflammatory responses.

A marked reduction in interleukin-6 levels further confirms suppression of pro-inflammatory cytokine activity and modulation of immune signaling pathways. These findings suggest that the tested formulation contributes not only to the reduction of inflammatory manifestations but also to the restoration of immune homeostasis.

Considering that inflammatory processes and immune imbalance play a key role in the pathogenesis of many gynecological disorders, including infectious and chronic inflammatory conditions, the investigated plant extract combination may be considered a promising adjunctive therapeutic agent. Its anti-inflammatory, cytokine-regulating, and immune-supporting properties indicate potential applicability as a complementary remedy in the комплекс management of gynecological diseases.

Thus, the studied herbal formulation may serve as a supportive natural component alongside conventional treatment strategies aimed at improving therapeutic outcomes in women’s reproductive health. Further preclinical and clinical investigations are required to confirm its efficacy, optimize dosage, and ensure long-term safety in gynecological applications.

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