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Original article

Dose-Dependent Effects of *Artemisia herba-alba* Extract on Hematological, Immunological, and Antioxidant Parameters in Male Rabbits

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Abstract
Artemisia herba-alba is a medicinal plant of considerable interest because of its potential biological and antioxidant properties. The present study evaluated the effects of *Artemisia herba-alba* administration at doses of 150 and 300 mg/kg on hematological, immunological, and antioxidant biomarkers in rabbits. The hematological profile showed gradual increases in RBC count, hemoglobin, and packed cell volume, with RBCs increasing from 5.82 ± 0.34 ×10^6/µL in controls to 6.10 ± 0.41 ×10^6/µL at 300 mg/kg, while hemoglobin increased from 12.4 ± 0.6 to 13.2 ± 0.7 g/dL. WBC counts increased significantly from 7.15 ± 0.52 ×10^3/µL in controls to 8.42 ± 0.61 ×10^3/µL and 9.18 ± 0.74 ×10^3/µL at 150 and 300 mg/kg, respectively ($p < 0.05$). Lymphocyte percentages increased from 62.0 ± 3.1% to 69.2 ± 3.4%, whereas neutrophils decreased from 31.5 ± 2.4% to 25.5 ± 2.2% at the high dose ($p < 0.05$). Immunological biomarkers also increased significantly, with IgG rising from 14.2 ± 1.1 to 19.1 ± 1.5 mg/mL and IgM from 1.85 ± 0.19 to 2.48 ± 0.23 mg/mL ($p < 0.05$). Antioxidant enzyme activities increased markedly, with SOD increasing from 42.5 ± 3.2 to 59.4 ± 4.6 U/mL and CAT from 24.1 ± 2.0 to 33.8 ± 2.8 µmol/min/mg. Conversely, MDA decreased significantly from 4.85 ± 0.42 to 3.10 ± 0.29 nmol/mL ($p < 0.05$). Overall, *Artemisia herba-alba* administration was associated with dose-related changes in hematological and immune parameters, enhanced antioxidant enzyme activities, and reduced MDA levels, with the most pronounced responses observed at 300 mg/kg.
Keywords: *Artemisia herba-alba*; Hematological Parameters; Immunoglobulins; Oxidative Stress; Rabbits.

Introduction

Medicinal plants have historically served as indispensable reservoirs of bioactive pharmacophores, offering therapeutic alternatives with multifaceted pharmacological activities [1]. Artemisia herba-alba Asso. (Asteraceae), locally designated as "Al-Shih," is widely distributed across North Africa, the Middle East, and the Mediterranean basin. Ethnomedicinally, it has been prescribed for the management of diabetes mellitus, gastrointestinal disorders, microbial infections, and inflammatory conditions. Phytochemically, Artemisia species are renowned for their complex secondary metabolite profiles, including sesquiterpene lactones (such as absinthin and anabsinthin), essential oils (thujone, camphor, and chrysanthenone), flavonoids, and phenolic acids. These constituents contribute significantly to their pronounced antioxidant, antimicrobial, and anti-inflammatory properties [2]. However, detailed empirical evaluations concerning their systemic immunomodulatory efficacy and comprehensive physiological safety margins, particularly on male reproductive and systemic organ functions in animal models, require rigorous scientific exploration [3].

Oxidative stress plays a fundamental role in the pathogenesis of numerous chronic and acute physiological dysfunctions. Endogenous antioxidant defense mechanisms are frequently insufficient to neutralize excessive reactive oxygen species (ROS), necessitating exogenous supplementation via bioactive botanical agents [4]. This study aims to elucidate the dose-dependent impact of Artemisia herba-alba extract administration on hematological profiles, lipid peroxidation indices, antioxidant enzyme activities, and immunological parameters in healthy male rabbits.

Materials and Methods

Aerial parts of Artemisia herba-alba were authenticated by departmental botanists, thoroughly washed with distilled water, shade-dried at room temperature, and pulverized into a uniform coarse powder. Extraction was executed via the cold maceration method using a hydroalcoholic solvent system (ethanol: water, 70:30 v/v) for 72 hours with intermittent agitation. The resulting extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40 °C. The dried crude extract was stored in airtight amber glass vials at 4 °C until experimental administration. A total of thirty healthy adult male

New Zealand White rabbits (Oryctolagus cuniculus), weighing approximately 2.0 ± 0.2 kg, were procured and housed under standard laboratory conditions (temperature 22 ± 2 °C, 55 ± 5% relative humidity, 12-hour light/dark cycle). Animals were provided with a standard pellet diet and clean drinking water ad libitum. Following a two-week acclimatization period, the rabbits were randomly allocated into three distinct experimental groups (n = 10 per

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group): Group I (Control): Received normal saline daily via oral gavage. Group II (Low Dose): Administered Artemisia herba-alba extract at a dose of 150 mg/kg body weight daily for 30 consecutive days. Group III (High Dose): Administered Artemisia herba-alba extract at a dose of 300 mg/kg body weight daily for 30 consecutive days.

At the termination of the 30-day experimental protocol, animals were fasted overnight and humanely euthanized. Blood samples were collected via cardiac puncture into EDTA-coated tubes for hematological evaluations and non-heparinized centrifuge tubes for serum separation. Serum was aspirated following centrifugation at 3000 rpm for 15 minutes and stored at -20 °C for subsequent biochemical and hormonal assays. Lipid peroxidation was assessed by measuring serum malondialdehyde (MDA) levels, while antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), were evaluated spectrophotometrically.

Immunological evaluation encompassed total leukocyte count (TLC), differential leukocyte count, phagocytic activity, and serum immunoglobulin G (IgG) and IgM quantification. Statistical Analysis: All quantitative data were expressed as mean ± standard deviation (SD) for each experimental group (n = 10). Data were first assessed for normality of distribution using the Shapiro–Wilk test and for homogeneity of variance using Levene's test.

Since these assumptions were satisfied, differences among the control, low-dose, and high-dose groups were evaluated using one-way analysis of variance (ANOVA). Where the overall ANOVA indicated a statistically significant difference, Tukey's Honestly Significant Difference (HSD) post-hoc test was applied for pairwise multiple comparisons between the control group and each treatment group, as well as between the two treatment groups themselves. Dose-dependent trends across the three groups were additionally examined using linear regression / Pearson correlation analysis where appropriate. All statistical computations were performed using SPSS software (Statistical Package for the Social Sciences, version 26.0, IBM Corp., Armonk, NY, USA) and cross-checked in GraphPad Prism (version 9.0). A probability value of $p < 0.05$ was considered statistically significant, and $p < 0.01$ was regarded as highly significant. Results are presented in tabular form as mean ± SD, with asterisks (*) denoting statistically significant differences relative to the control group.

Results

Table 1 presents the hematological profile of rabbits treated with *Artemisia herba-alba* at doses of 150 and 300 mg/kg compared with the control group. RBC count showed a gradual increase from 5.82 ± 0.34 $\times 10^6$/µL in the control group to 5.95 ± 0.28 $\times 10^6$/µL at 150 mg/kg and 6.10 ± 0.41 $\times 10^6$/µL at 300 mg/kg. Hemoglobin concentration similarly increased from 12.4 ± 0.6 g/dL in controls to 12.8 ± 0.5 g/dL and 13.2 ± 0.7 g/dL, respectively. PCV increased from 37.5 ± 1.8% in the control group to 38.2 ± 1.5% at the low dose and 39.4 ± 2.1% at the high dose. A more pronounced change was observed in the leukocyte profile. WBC counts increased significantly from 7.15 ± 0.52 $\times 10^3$/µL in the control group to 8.42 ± 0.61 $\times 10^3$/µL at 150 mg/kg and 9.18 ± 0.74 $\times 10^3$/µL at 300 mg/kg **(**$p < 0.05$**).** Lymphocyte percentages increased from 62.0 ± 3.1% in controls to 66.5 ± 2.8% at the low dose and 69.2 ± 3.4% at the high dose, with the high-dose group showing a statistically significant difference **(**$p < 0.05$**).** In contrast, neutrophils decreased from 31.5 ± 2.4% in the control group to 28.0 ± 2.1% and 25.5 ± 2.2% following treatment with 150 and 300 mg/kg, respectively, with the high-dose group showing a significant reduction **(**$p < 0.05$**).**

Table 1. Hematological Profile of Control and Artemisia herba-alba-Treated Rabbits

Parameter	Control Group	Low Dose (150 mg/kg)	High Dose (300 mg/kg)
Red Blood Cells (RBCs, $\times 10^6$/µL)	5.82 ± 0.34	5.95 ± 0.28	6.10 ± 0.41
Hemoglobin (Hb, g/dL)	12.4 ± 0.6	12.8 ± 0.5	13.2 ± 0.7
Packed Cell Volume (PCV, %)	37.5 ± 1.8	38.2 ± 1.5	39.4 ± 2.1
White Blood Cells (WBCs, $\times 10^3$/µL)	7.15 ± 0.52	8.42 ± 0.61 *	9.18 ± 0.74 *
Lymphocytes (%)	62.0 ± 3.1	66.5 ± 2.8	69.2 ± 3.4 *
Neutrophils (%)	31.5 ± 2.4	28.0 ± 2.1	25.5 ± 2.2 *

*Values are expressed as Mean ± SD; * $p < 0.05$ indicates statistical significance compared to the control group.*

Table 2 presents the effects of *Artemisia herba-alba* administration at 150 and 300 mg/kg on selected immunological and antioxidant biomarkers in rabbits. Serum IgG concentration increased significantly from 14.2 ± 1.1 mg/mL in the control group to 16.8 ± 1.3 mg/mL at 150 mg/kg and 19.1 ± 1.5 mg/mL at 300 mg/kg ($p < 0.05$). Similarly, serum IgM increased from 1.85 ± 0.19 mg/mL in controls to 2.20 ± 0.21 mg/mL and 2.48 ± 0.23 mg/mL following administration of the low and high doses, respectively ($p < 0.05$). The antioxidant profile showed a consistent dose-related increase in enzymatic antioxidant activity. SOD activity increased significantly from 42.5 ± 3.2 U/mL in the control group to 51.8 ± 4.0 U/mL at 150 mg/kg and 59.4 ± 4.6 U/mL at 300 mg/kg ($p < 0.05$). Likewise, CAT

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activity increased from 24.1 ± 2.0 µmol/min/mg in controls to 29.5 ± 2.3 µmol/min/mg at the low dose and 33.8 ± 2.8 µmol/min/mg at the high dose ($p < 0.05$).

In contrast, MDA concentrations demonstrated a significant dose-dependent reduction, decreasing from 4.85 ± 0.42 nmol/mL in the control group to 3.92 ± 0.35 nmol/mL at 150 mg/kg and 3.10 ± 0.29 nmol/mL at 300 mg/kg ($p < 0.05$). Thus, all measured immunological and antioxidant biomarkers exhibited statistically significant changes relative to the control group. The overall pattern was characterized by increased IgG, IgM, SOD, and CAT, accompanied by decreased MDA, with the magnitude of change generally greater at 300 mg/kg than at 150 mg/kg.

Table 2. Immunological and Antioxidant Biomarkers

Parameter	Control Group	Low Dose (150 mg/kg)	High Dose (300 mg/kg)
Serum IgG (mg/mL)	14.2 ± 1.1	16.8 ± 1.3 *	19.1 ± 1.5 *
Serum IgM (mg/mL)	1.85 ± 0.19	2.20 ± 0.21 *	2.48 ± 0.23 *
Superoxide Dismutase (SOD, U/mL)	42.5 ± 3.2	51.8 ± 4.0 *	59.4 ± 4.6 *
Catalase (CAT, µmol/min/mg)	24.1 ± 2.0	29.5 ± 2.3 *	33.8 ± 2.8 *
Malondialdehyde (MDA, nmol/mL)	4.85 ± 0.42	3.92 ± 0.35 *	3.10 ± 0.29 *

*Values are expressed as Mean ± SD; * $p < 0.05$ indicates statistical significance compared to control.*

Discussion

The present study demonstrated that *Artemisia herba-alba* administration produced favorable changes in hematological, immunological, and antioxidant parameters in rabbits. The observed increases in RBCs, hemoglobin, and packed cell volume indicate preservation of the erythrocyte profile and suggest that treatment did not adversely affect the major hematological indices. These findings are consistent with the recognized biological activity of *A. herba-alba*, which contains phenolic compounds, flavonoids, and terpenoid constituents with potential antioxidant and biological effects. The significant increase in total WBC count and lymphocyte percentage, accompanied by a reduction in neutrophil percentage, indicates an alteration in leukocyte distribution following treatment. The increase in lymphocytes may reflect an enhanced immune response, while the reduction in neutrophil proportion indicates a shift in the relative leukocyte profile [5].

Similar immunomodulatory properties have been reported for several *Artemisia* species and have been associated with their diverse phytochemical constituents. The significant increases in IgG and IgM further support the immunological response observed in the treated animals [6]. The stronger response at the higher dose suggests a dose-related effect of *A. herba-alba* on humoral immune parameters.

Phenolic and flavonoid compounds present in the plant may contribute to this biological activity, although further studies involving cytokines, lymphocyte subsets, and immune-cell function are required to clarify the underlying mechanisms. The antioxidant findings represent an important aspect of the present study [7]. Treatment resulted in increased SOD and CAT activities, indicating enhancement of enzymatic antioxidant defense. SOD plays a central role in the detoxification of superoxide radicals, whereas CAT contributes to the elimination of hydrogen peroxide. The simultaneous elevation of these antioxidant enzymes suggests improved endogenous antioxidant capacity following *A. herba-alba* administration. The reduction in MDA provides additional evidence of an improved oxidative status. Since MDA is widely used as a marker of lipid peroxidation, its decrease together with increased SOD and CAT activities indicates a coordinated antioxidant response and reduced lipid oxidative damage [8]. This pattern agrees with previous reports describing substantial antioxidant activity of *A. herba-alba* extracts and their phenolic-rich fractions [9,10].

Study Limitations

This study has several limitations that should be acknowledged. First, organ weights, hepatic and renal biochemical markers, and histopathological examinations were not evaluated, so the systemic safety profile of the extract beyond the parameters reported here remains to be established. Second, the relatively small sample size (n = 10 per group) and the absence of a phytochemical fingerprint (e.g., HPLC quantification of the extract's active constituents) limit the ability to attribute the observed effects to specific compounds. Third, only two dose levels were tested over a single 30-day period, so the dose-response relationship at higher doses, potential toxicity thresholds, and effects following long-term or intermittent administration were not assessed. Finally, cytokine profiling and lymphocyte subset analysis, which would clarify the mechanistic basis of the observed immunomodulatory effects, were beyond the scope of this study. Future work should address these aspects to more comprehensively characterize the safety and mechanism of action of Artemisia herba-alba extract.

Conclusion

The present study demonstrates that Artemisia herba-alba administration produced beneficial dose-related changes in hematological, immunological, and antioxidant

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parameters in rabbits. Treatment enhanced antioxidant defense, as reflected by increased SOD and CAT activities, while reducing MDA levels. The observed improvements in immunoglobulin concentrations and leukocyte profiles further indicate potential immunomodulatory activity. Overall, A. herba-alba shows promising biological activity and may represent a valuable natural source of antioxidant and immunomodulatory compounds, warranting further investigation of its active constituents, mechanisms of action, and long-term safety.

Conflict of interest. Nil

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