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EXPERIMENTAL EVALUATION OF THE EFFECTS OF A HERBAL PHYTOCOMPOSITION ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN AN LPS-INDUCED BRONCHITIS MODEL

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Annotation. The study aimed to comparatively evaluate three phytocompositions prepared from *Cyperus rotundus* L., *Urtica dioica* L., and *Cinnamomum cassia* Blume in a lipopolysaccharide (LPS)-induced experimental bronchitis model, identify the most effective sample, and compare its effects on hematological and biochemical parameters with those of ambroxol. The scientific novelty of the study lies in the comprehensive evaluation of three compositional variants under identical experimental conditions and the direct experimental selection of the most effective sample. Adult male albino laboratory rats weighing 200±20 g were used (n=5 in each group). Bronchitis was induced by intranasal administration of LPS obtained from the *Escherichia coli* O111 strain. Model establishment was confirmed after 48 hours using peripheral blood parameters. The effects of the three samples were studied for five days, with ambroxol used as the reference drug. Results were expressed as M±m and statistically analyzed using Student's t-test and one-way analysis of variance; $p<0.05$ was considered statistically significant. LPS increased the white blood cell count (WBC) from 20.6±1.8×10⁹/L to 27.2±0.81×10⁹/L. After five days of treatment with sample No. 3, WBC decreased to 12.8±1.6×10⁹/L; the red blood cell count (RBC) was 11.9±0.12×10¹²/L, hemoglobin concentration (HGB) was 218±3.01 g/L, and hematocrit (HCT) was 0.68±0.004. Alanine aminotransferase (ALT) activity was 51.9±4.0 U/L, aspartate aminotransferase (AST) was 61.3±2.8 U/L, alkaline phosphatase was 339±3.5 U/L, and total bilirubin was 2.86±0.4 µmol/L. Results obtained under identical experimental conditions demonstrated that sample No. 3 produced the most pronounced reduction in inflammation-related leukocyte changes and normalization of selected erythrocyte parameters among the tested phytocompositions, while some biochemical findings approached the values observed in the ambroxol group.

Keywords: lipopolysaccharide, experimental bronchitis, herbal phytocomposition, *Cyperus rotundus* L., *Urtica dioica* L., *Cinnamomum cassia* Blume, hematological parameters, biochemical parameters, Ambroxol.

How to Cite

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Introduction. Inflammatory processes of the respiratory tract are accompanied by local and systemic alterations that may be reflected in the cellular composition of peripheral blood and selected biochemical parameters. Lipopolysaccharide (LPS)-induced experimental models are used to investigate such inflammatory responses and to comparatively evaluate the effects of tested substances under controlled conditions. Changes in leukocyte counts and individual peripheral blood cell fractions following LPS exposure represent important experimental indicators for assessing the development of the inflammatory process [1,2].

In recent years, increasing attention has been paid to plant materials rich in biologically active compounds and to multicomponent phytocompositions developed from such materials. Combining different plant materials within a single composition makes it possible to utilize their chemical constituents and

functional properties in a complex system. In this context, *Cyperus rotundus* L., *Urtica dioica* L., and *Cinnamomum cassia* Blume are of scientific and practical interest. Various biologically active constituents present in these plant materials may contribute to the functional properties of multicomponent phytocompositions [3,4].

A comprehensive set of laboratory parameters is useful for the experimental evaluation of phytocompositions. Determination of lymphocytes, granulocytes, red blood cell count (RBC), blood hemoglobin concentration (HGB), hematocrit value (HCT), and platelet count (PLT) and other hematological indices enables comparative assessment of changes occurring in experimental animals. At the same time, analysis of serum total protein, albumin, ALT, AST, alkaline phosphatase, and total bilirubin provides additional information on biochemical changes during the experimental period [5,6].

Therefore, the present study comparatively investigated the effects of three plant-based samples under conditions of an LPS-induced bronchitis model.

Materials and methods

The study was conducted on adult male white laboratory rats weighing 200±20200\pm20 g. Before the experiment, the animals' general condition, locomotor activity, and external appearance were examined. The animals were balanced according to body weight and randomly allocated to the experimental groups. Each group consisted of five animals (n=5n=5). Throughout the experiment, the rats were maintained under standard vivarium conditions and had free access to standard laboratory feed and drinking water. All procedures involving the maintenance and experimental use of animals were performed in accordance with generally accepted bioethical principles for the use of laboratory animals in scientific research [7,8].

Three different plant compositions were evaluated for their effects on haematological and biochemical parameters in a model of LPS-induced bronchitis. Sample No. 1 consisted of *Lecanora rupicola* lichen, licorice, and the aerial parts of mint combined at a ratio of 1:2:1. Sample No. 2 was prepared from *Lobothallia lacteola* lichen, rosehip, and the aerial parts of mint at a ratio of 1:1:1. Sample No. 3 consisted of the rhizomes of *Cyperus rotundus* L., the aerial parts of *Urtica dioica* L., and *Cinnamomum cassia* Blume combined at a ratio of 2:1:1. Ambroxol tablets containing 30 mg of the active substance and manufactured by Arterium Corporation were used as the reference preparation.

Experimental bronchitis was induced using lipopolysaccharide (LPS) obtained from *Escherichia coli* O111. LPS was dissolved in sterile 0.9% sodium chloride solution to obtain a final concentration of 2 mg/mL. The prepared LPS suspension was administered intranasally to the rats at a dose of 1 mg/kg. Depending on the body weight of the animals, each rat received approximately 0.20–0.21 mg of LPS in a total suspension volume of 100–105 μL. The suspension was divided into two equal portions and slowly instilled into the nostrils at approximately 50 μL per nostril.

Following LPS administration, the animals were monitored individually. Their general condition, locomotor activity, respiratory characteristics, food and water consumption, and external appearance were assessed. Decreased locomotor activity and deterioration of the general condition, which are characteristic manifestations of an inflammatory response, were observed following LPS exposure. Forty-eight hours after LPS administration, peripheral blood samples were collected, and the development of the experimental bronchitis model was evaluated based on haematological parameters.

After confirmation of the experimental model, the animals were divided into six groups: healthy, control, Sample No. 1, Sample No. 2, Sample No. 3, and Ambroxol groups. The healthy group consisted of intact animals that did not receive LPS. The control group consisted of animals that received LPS but were not treated with the investigated plant compositions. The animals in the experimental groups received Samples No. 1, No. 2, and No. 3, respectively, while the reference group received Ambroxol. The effects of the investigated samples and the reference preparation were evaluated over five consecutive days.

The haematological examination included the determination of the total leukocyte count (WBC), lymphocytes (Lym), granulocytes (Gran), mid-sized cells (Mid), erythrocytes (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean

corpuscular haemoglobin concentration (MCHC), and platelets (PLT). Lym%, Gran%, and Mid% represented the relative proportions of the respective cells, whereas Lym#, Gran#, and Mid# represented their absolute counts. Haematological analyses were performed in accordance with standard laboratory procedures.

After five days of treatment, biochemical blood parameters were examined to assess not only the effects of the plant compositions on the inflammatory response but also their possible influence on the functional state of the liver. Total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and total bilirubin were determined in the blood serum. Biochemical analyses were performed using highly sensitive diagnostic reagent kits manufactured by Cypress Diagnostics, in accordance with the manufacturer's instructions and standard laboratory protocols.

Considering the exploratory nature of the study and the principle of reducing the number of animals used in experimental research, five animals were included in each group. This sample size was used to identify the principal haematological and biochemical changes induced by LPS and to provide a preliminary evaluation of the directional effects of the investigated samples. The small sample size was taken into account as a methodological limitation when interpreting the results.

The results were expressed as the arithmetic mean and the standard error of the mean, M±mM\pm m. Statistical calculations were performed using Microsoft Excel. Differences between the healthy, control, and experimental groups were evaluated using Student's tt-test. Differences were considered statistically significant at $p<0.05p<0.05$ and highly significant at $p<0.01p<0.01$ and $p<0.005p<0.005$. A value of $p<0.09p<0.09$ was interpreted as a trend toward statistical significance. In interpreting the results, each experimental group was primarily compared with the LPS-treated control group.

Results and discussion

To confirm the establishment of the experimental bronchitis model, peripheral blood parameters were examined in rats from the healthy and control groups 48 h after the intranasal administration of LPS. Each group consisted of five male white laboratory rats ($n=5n=5$). The animals in the healthy group did not receive LPS, whereas the animals in the control group were intranasally administered LPS obtained from *Escherichia coli* O111 at a dose of 1 mg/kg. The results are presented in Table 1.

Table 1 Peripheral blood cell composition of rats 48 h after LPS administration (M±m, n=5)

Parameters	Healthy group	Control
WBC, 10^9/L	20.6±1.8	27.2±0.81*
Lym, %	0.82±0.03	0.84±0.14
Gran, %	0.07±0.02	0.08±0.009
Mid, %	0.09±0.01	0.08±0.05
Lym#, 10^9/L	17.1±2.16	19.8±2.76
Gran#, 10^9/L	1.5±0.27	1.90±0.53*
Mid#, 10^9/L	2.0±0.015	1.82±0.36
RBC, 10^{12}/L	11.5±0.3	11.4±0.19
HGB, g/L	210.5±8.5	193±10.6
HCT	0.65±0.03	0.60±0.03
MCV, fL	56.2±1.0	54.6±0.94
MCH, pg	18.1±0.25	17.4±0.12

MCHC, g/L	323±1.0	319±2.85
PLT, 10^9/L	524±176	718±64.5

*Note: *p<0.05 — statistically significant difference compared with the healthy group.*

At 48 h after LPS administration, the WBC count in the control group increased from $20.6\pm1.8\times10^9$/L in the healthy group to $27.2\pm0.81\times10^9$/L ($p<0.05p<0.05$). Thus, the total leukocyte count increased by approximately 32%:

$$\Delta WBC = \frac{27.2 - 20.6}{20.6} \times 100\% = 32.0\%$$

At the next stage, the effects of Sample 1, Sample 2, and Sample 3 were evaluated over five days. The results were compared with those of the healthy and control groups and the Ambroxol reference group (Table 2).

Table 2. Effects of the samples on peripheral blood cell composition in rats with LPS-induced bronchitis after 5 days of treatment (M±m, n=5)

Parameters	**Healthy**	**Control**	**No. 1**	**No. 2**	**No. 3**	**Ambroxol**
WBC, 10^9/L	20.6±1.8	26.6±3.7	20.0±2.4	14.8±2.1	12.8±1.6#	14.8±1.6
Lym, %	0.82±0.03	0.78±0.03	0.79±0.01	0.79±0.02	0.77±0.03	0.76±0.06
Gran, %	0.07±0.02	0.13±0.02#	0.10±0.02	0.10±0.005	0.14±0.04	0.15±0.06
Mid, %	0.09±0.01	0.086±0.01	0.10±0.005	0.10±0.02	0.09±0.01	0.09±0.004
Lym#, 10^9/L	17.1±2.16	17.2±3.48	15.8±1.67	11.8±1.99	9.87±1.6#	11.4±2.17
Gran#, 10^9/L	1.5±0.27*	2.65±0.37	2.06±0.6	1.53±0.14*	1.74±0.27#	2.08±0.78
Mid#, 10^9/L	2.0±0.015	1.78±0.16	2.14±0.15	1.46±0.04	1.17±0.24	1.33±0.2
RBC, 10^{12}/L	11.5±0.3***	9.94±0.19	10.5±1.0	10±0.28	11.9±0.12***	9.73±0.45
HGB, g/L	210.5±8.5*	183.6±5.45	195.5±15	188±4.5	218±3.01***	173.5±1.5
HCT	0.65±0.03*	0.56±0.02	0.58±0.06	0.56±0.005	0.68±0.004***	0.54±0.01
MCV, fL	56.2±1.0	56.5±1.6	55±0.8	56.8±2.15	56.7±0.2	55.1±1.4
MCH, pg	18.1±0.25	18.5±0.712	18.6±0.3	18.8±0.95	18.1±0.05	17.9±1.0
MCHC, g/L	323±1.0	326.6±7.2	338.5±10.5	331±5.0	321±2.0	324.5±9.6
PLT, 10^9/L	524±176	687±119	628±55.1	725±53.6	513±112	608±6.5

*Note: *p<0.05;* *p<0.01; ***p<0.005 — statistically significant differences compared with the control group; #p<0.09 — a trend toward a difference compared with the control group.*

After the five-day experiment, the most pronounced changes among the investigated samples were observed in the Sample 3 group. The WBC count was $26.6\pm3.7\times10^9$/L in the control group and decreased

to 12.8±1.6×10⁹/L in the Sample 3 group. In the Ambroxol group, the WBC count was 14.8±1.6×10⁹/L. In the Sample 3 group, Lym# and Gran# were 9.87±1.6×10⁹/L and 1.74±0.27×10⁹/L, respectively.

$$\Delta WBC = \frac{27.2 - 20.6}{20.6} \times 100\% = 32\%$$

Leukocytosis is one of the principal haematological signs indicating the development of a systemic inflammatory response to LPS. The absolute granulocyte count (Gran#) increased from 1.50±0.27×10⁹/L in the healthy group to 1.90±0.53×10⁹/L in the control group ($p<0.05p<0.05$). This increase was approximately 26.7%:

$$\Delta Gran\# = \frac{1.90 - 1.50}{1.50} \times 100\% = 26.7\%$$

Granulocytes, particularly neutrophils, are key cells of the innate immune response and are among the first cells to migrate to sites of acute inflammation. Therefore, the increase in the absolute granulocyte count (Gran#) confirms the development of an acute inflammatory response in the respiratory tract following LPS exposure.

The Lym parameter represents the relative proportion of lymphocytes in the total leukocyte count, whereas Lym# indicates their absolute count in peripheral blood. In the control group, the Lym# value increased from 17.1±2.16×10⁹/L to 19.8±2.76×10⁹/L.

Thus, at 48 h after LPS administration, the total leukocyte count (WBC) and absolute granulocyte count (Gran#) increased significantly. These findings confirm the development of an LPS-induced inflammatory response and the establishment of the experimental bronchitis model in rats. Changes in the other haematological parameters were not statistically significant and were therefore interpreted with caution.

The inclusion of five animals in each group was related to the exploratory nature of the study. The small sample size may have limited the statistical power and prevented some biologically relevant changes from reaching statistical significance. Therefore, the findings should be considered preliminary experimental evidence and require confirmation in further studies with a larger sample size.

To complement the hematological findings, serum biochemical parameters were evaluated after the five-day experimental period (Table 3).

Table 3 Effects of the samples on serum biochemical parameters in rats with LPS-induced bronchitis after 5 days of treatment (M±m, n=5)

Groups	Total protein, g/L	Albumin, g/L	ALT, U/L	AST, U/L	Alkaline phosphatase, U/L	Total bilirubin, mmol/L
Healthy	68.3±5.4	33.0±1.5	62.2±5.1	55.8±2.7	346±11	3.29±0.2
Control	68.7±2.8	38.7±3.1	77.7±5.4	69.5±3.9	363±12	3.60±0.4
No. 1	65.1±1.6	40.6±4.7	85.7±8.2	83.4±7.4*	351±3.1	2.99±0.5
No. 2	65.2±3.9	41.8±1.8	82.8±1.5	92.1±6.7**	350±3.2	3.51±0.5
No. 3	69.6±1.8	41.3±3.2	51.9±4.0*	61.3±2.8	339±3.5	2.86±0.4
Ambroxol	64.3±2.4	39.4±1.3	52.5±3.9*	57.7±3.8	346±4.4	2.99±0.1

*Note: *p<0.05;* *p<0.01 — statistically significant differences compared with the control group.*

No statistically significant differences from the control group were observed for total protein, albumin, alkaline phosphatase, or total bilirubin ($p>0.05$). However, ALT activity in the Sample 3 group decreased from 77.7±5.4 U/L in the control group to 51.9±4.0 U/L, and the difference was statistically significant ($p<0.05$). ALT activity in the Ambroxol group was 52.5±3.9 U/L.

AST activity was 61.3±2.8 U/L in the Sample 3 group and 57.7±3.8 U/L in the Ambroxol group. In contrast, AST values in the Sample 1 and Sample 2 groups were 83.4±7.4 U/L and 92.1±6.7 U/L, respectively. Total bilirubin in the Sample 3 group was 2.86±0.4 mmol/L, while alkaline phosphatase activity was 339±3.5 U/L.

Overall, the combined evaluation of hematological and biochemical findings showed that Sample 3 produced the most pronounced changes among the three investigated samples. In particular, changes in WBC and erythrocyte-related parameters, together with the statistically significant reduction in ALT activity, represent the main experimental findings characterizing the effect of Sample 3 under the conditions of the LPS-induced model. Since no statistically significant differences were observed for the remaining biochemical parameters, stronger biological interpretations of these findings should be made with caution.

Conclusion. The study results demonstrated that an experimental bronchitis model was successfully established in rats using lipopolysaccharide (LPS). In accordance with the stated objective, three phytocompositions prepared from *Cyperus rotundus* L., *Urtica dioica* L., and *Cinnamomum cassia* Blume were comparatively evaluated under identical experimental conditions for their effects on hematological and biochemical parameters. The most effective sample was identified, and its effects were compared with the findings obtained in the ambroxol group.

Among the examined phytocompositions, sample No. 3, prepared at a ratio of 2:1:1, demonstrated the most pronounced positive effect. This sample reduced inflammation-associated leukocyte alterations, supported the recovery of selected erythrocyte parameters, and brought certain biochemical indicators closer to the values observed in the healthy and ambroxol groups. The effects of samples No. 1 and No. 2 were less pronounced than those of sample No. 3.

Thus, the objective of the study was achieved: the three phytocompositions were comparatively evaluated, sample No. 3 was identified as the most effective composition, and some indicators of its activity were found to be comparable with the results obtained for ambroxol. These findings provide a scientific basis for further in-depth investigation of phytocomposition No. 3 as a promising formulation for developing an adjunct plant-based product intended for use in inflammatory conditions of the respiratory tract.

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