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HAEMATOLOGICAL ASPECTS OF INFLAMMATORY ANAEMIA IN MICE

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Abstract: *The research was conducted on 10 CD1 laboratory mice, non-consanguineous strains, divided into two groups of 5 individuals per group. The body weight of individuals ranged from 20 to 30 grams. Both groups were given the same favourable climate, humidity and light conditions. The diet consisted of pelleted rodent feed, with feed and water administered ad libitum. The control group was injected subcutaneously, at the beginning of the experimental period, with 1 ml NaCl and the experimental group with 0.8 ml turpentine. The duration of the experiment was 14 days. At the end of the experiment, decreases of the erythrocyte count (RBC), haemoglobin, haematocrit, derived erythrocyte constants and increases of the lymphocyte count (WBC) were observed in the experimental group - injected with turpentine essence. The leukocyte formula (differential blood count) showed increases in all leukocyte categories in the experimental group.*
Keywords: *turpentine, anaemia, inflammation, mouse.*

• Introduction

The inflammatory reaction can be defined as a complex of local and general reactions of the body in response to the action of different pathogens. This complex may include alterative, aggressive and proliferative phenomena (3, 9, 11, 12).
The inflammatory reaction is defensive, as the mechanism of action attempts to remove the pathogen, in order to heal, but it can become harmful and develop into a pathological state (2).

For this experiment we used turpentine as a pro-inflammatory substance. Turpentine, by inducing an inflammatory response, leads to hepcidin synthesis (12, 15). Hepcidin is a 25 aminoacid peptide hormone produced mainly by hepatocytes, and represents the main regulator of iron homeostasis both in the body's physiological state and during inflammation (10, 13, 14). Excess production of this hormone causes iron sequestration in macrophages and hypovolaemia (18, 20, 21).
Hepcidin acts by binding and inactivating ferroportin, which delivers iron into the plasma from all iron-carrying cells. In a classical endocrine feedback system, hepcidin production is stimulated by plasma iron and iron stores.

Pathologically elevated hepcidin concentrations are seen in iron-refractory iron-deficiency anaemia, in anaemia of inflammatory cause, and in anaemias established associated with chronic kidney disease, where increased hepcidin limits the availability of iron for erythropoiesis (22).

• Material and method

The experiment was carried out in the Biobase of the Faculty of Veterinary Medicine in Bucharest, on two groups of laboratory mice, a control group and an experimental group. The mice were CD1 non-consanguineous strains and were divided into 5 individuals per group.

The pro-inflammatory substance used was **turpentine**.

The individuals from both groups had body weights between 20 and 30 grams and were kept under favourable climate, humidity, and lighting conditions at all times.

Feed was administered ad libitum and consisted of combined granulated rodent feed, also water was administered at discretion.

Mice in the control group were injected subcutaneously with 1 ml NaCl 0.9% at the beginning of the experiment and mice in the experimental group were injected with 0.8 ml turpentine subcutaneously.
The duration of the experiment was 14 days.

On the last day of the experiment, blood sample were harvested. Erythrocyte count (RBC), leukocyte count (WBC), hemoglobin, hematocrit, and derived erythrocyte constants were determined for each individual in both groups.

The haematological investigations were performed using a 5 DIFF LaserCyte haematology analyser from Idexx Laboratory.

The differential blood count (leukocyte formula) was performed by the classical method, analysing the blood smears under the microscope. The smear staining was performed by the May Grunwald Giemsa method.

Interpretation of the results was performed using the classic T test.

• Results and discussions

In the experimental group, the decrease of the RBC, haemoglobin, haematocrit, MCV, MCH, and MCHC mean values was observed, along with the increase of the WBC mean value.

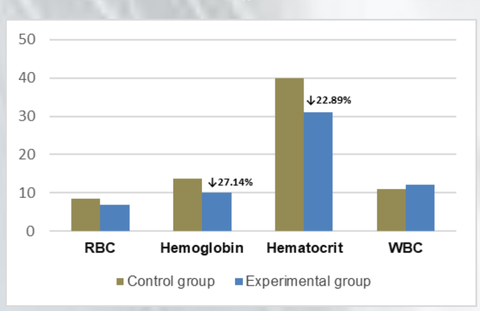
The RBC value showed statistically significant differences ($p<0.05$) between the control and experimental groups. The mean RBC value in the experimental group was 17.9% lower than the mean of the control group.
The mean values of the haematological parameters for the two groups are presented below, in Table 1 and the graphs from Fig. 1 and Fig. 2.

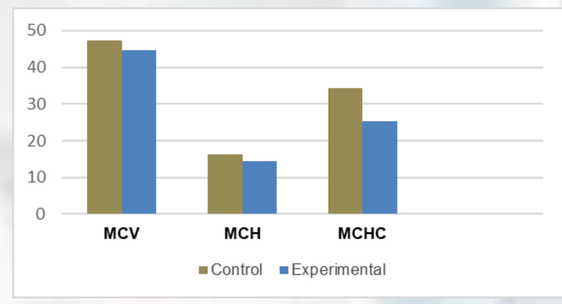
Table 1

Mean values of haematological parameters in the two studied groups – comparative analysis

Parameter	Control Group	Experimental Group	Percentage difference (%)
E x 10^6 / µl	8.48	6.96	**↓ 17.9***
Hb g/dl	13.78	10.04	**↓27.14***
HTC %	40.02	31	**↓22.89***
MCV μ^3	47.37	44.57	**↓6.28***
MCH pg Hb/E	16.26	14.42	**↓11.32***
MCHC g Hb/dl E	34.30	32.37	**↓5.63***
Leucocyte x 10^3/µl	11.02	12.04	**↑9.26**

*P<0.05 – significant differences



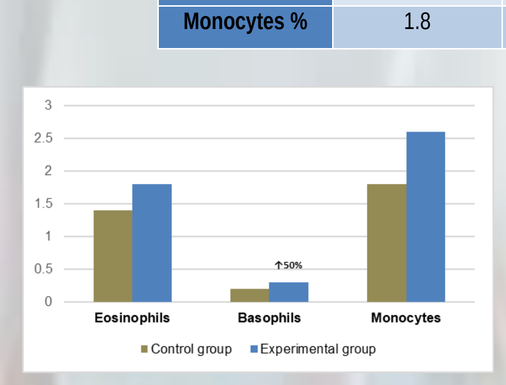


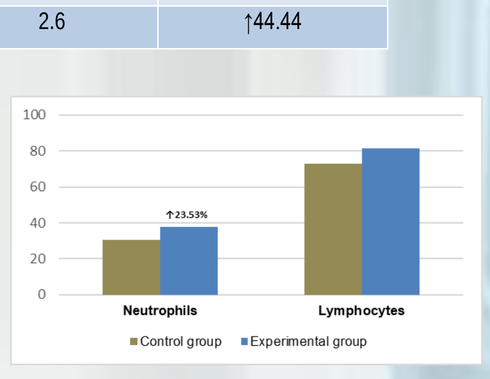
The differential blood count showed that all categories of white blood cells increased (Table 2, Fig. 3 and Fig. 4).

Following the administration of turpentine essence to the individuals in the experimental group, was observed an increase of the neutrophils percentage by 23.53%, eosinophils by 28.57%, basophils by 50%, lymphocytes by 11.78% and monocytes by 44.44% compared to the control group

The differential blood count mean values in the studied groups

WBC category	Control group	Experimental group	Percentage difference
Neutrophils %	30.6	37.8	↑23.53
Eosinophils %	1.4	1.8	↑28.57
Basophils %	0.2	0.3	↑50
Lymphocytes %	73	81.6	↑9.26
Monocytes %	1.8	2.6	↑44.44





• Conclusions

The haematological investigations carried out on both groups of mice showed that injection with turpentine in the experimental group resulted in a statistically significant decrease of most haematological parameters, namely, RBC by 17.9%, haemoglobin by 27.14%, and haematocrit by 22.89%. The WBC was higher in the experimental group by 9.26%.

The derived erythrocyte constants (MCV, MCH and MCHC) also decreased in the experimental group of mice, by 6.28%, 11.32% and 5.63%.

The differential blood count results showed increases in all white blood cell categories in the turpentine injected group: neutrophils percentage increased by 25.53%, eosinophils percentage increased by 28.57%, basophils percentage by 50%, lymphocytes percentage by 11.78% and monocytes percentage by 44.44%.

Therefore, based on the haematological modifications recorded in the experimental group, the diagnosis of inflammatory anaemia can be made, as a result of the proinflammatory effects of turpentine.

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