http://dx.doi.org/10.1590/1678-4162-13022

Arq. Bras. Med. Vet. Zootec., v.75, n.6, p.1029-1038, 2023

Hematological effects in acute toxicity generated by the administration of honeybee (*Apis mellifera* L.) venom in European rabbit (*Oryctolagus cuniculus*)

[*Efeitos hematológicos na toxicidade aguda gerada pela administração de veneno de abelha (Apis mellifera L.) em coelho europeu (Oryctolagus cuniculus)*]

G. *Pavel*[1], D. *Bratu*[2], D. *Bejenariu*[2], A.C. *Grădinaru*[1*]

[1]"Ion Ionescu de la Brad" University of Life Sciences, Faculty of Veterinary Medicine – 8, Mihail Sadoveanu Alley, 700489, Iasi, Romania
[2]Veterinary Clinic, Triovet, Botoșani, Romania

ABSTRACT

Various studies demonstrated risks and benefits in the use of bee venom, a natural product characterized by a complex organic structure. In this study, we aimed to test the effects of bee venom administration in rabbits. Hematological investigations were carried out by determining specific blood indicators at different time intervals and at varying doses of venom. The obtained results showed a toxic action dependent on the dose and the daily cumulative effect; in the case of erythrocyte and platelet parameters, bee venom progressively generated the onset of thrombocytosis and reduced hemolysis for the short term. In the case of leukocytes, the administration of bee venom caused an increase of WBC count, segmented neutrophils and Ne/Lymph ratio, proportional to the dose and time of bee venom inoculation, but within the reference range; the average values of unsegmented neutrophils and basophils was above the upper limit of the normal range, with oscillating changes during the experiment; monocytosis and eosinophilia was also noted at different doses and times of venom inoculation. The investigations carried out 7 days after stopping the administration of bee venom revealed, in many cases, a progressive return to normal for the hematological parameters, except blood platelets.

Keywords: bee venom, blood, toxicity, risk, benefits

RESUMO

Vários estudos demonstraram riscos e benefícios no uso do veneno de abelha, um produto natural caracterizado por uma estrutura orgânica complexa. Neste estudo, objetivou-se testar os efeitos da administração de veneno de abelha em coelhos. Investigações hematológicas foram realizadas, determinando-se indicadores sanguíneos específicos em diferentes intervalos de tempo e em diferentes doses de veneno. Os resultados obtidos mostraram uma ação tóxica dependente da dose e do efeito cumulativo diário; no caso dos parâmetros eritrocitários e plaquetários, o veneno de abelha gerou progressivamente o aparecimento de trombocitose e reduziu a hemólise em curto prazo. No caso dos leucócitos, a administração de veneno de abelha causou aumento na contagem de leucócitos, neutrófilos segmentados e relação Ne/Linfa, proporcional à dose e ao tempo de inoculação do veneno de abelha, mas dentro do intervalo de referência; os valores médios de neutrófilos e basófilos não segmentados estavam acima do limite superior da normalidade, com variações oscilantes durante o experimento; monocitose e eosinofilia também foram observadas em diferentes doses e tempos de inoculação do veneno. As investigações realizadas sete dias após a interrupção da administração do veneno de abelha revelaram, em muitos casos, um retorno progressivo ao normal dos parâmetros hematológicos, exceto plaquetas sanguíneas.

Palavras-chave: veneno de abelha, sangue, toxicidade, risco, benefícios

*Corresponding authors: gpavel@uaiasi.ro; a.c.gradinaru@uaiasi.ro
Submitted: March 30, 2023. Accepted: July 18, 2023.

INTRODUCTION

Bee venom is a complex organic substance, with an important content of proteins, phospholipids, and sugars. Various enzymes are included, such as phospholipase A_2, B, acid phosphomonoesterase, hyaluronidase, phosphatase, lysophospholipase, small proteins and peptides, such as melittin, apamin, adolapin, teeriapin, secapin, physiologically active amines, such as histamine, dopamine, noradrenaline, and amino acids. The major component of the dry venom is represented by melittin, which accounts for 40-50% of its composition, with 26 amino acids in its chemical structure. Among the sugars, the presence of glucose and fructose is noted. The bee venom also contains pheromones and minerals, such as Ca and Mg (El-Wahed *et al.,* 2019; Khalil *et al.,* 2021; Pak, 2017; Rybak-Chmielewska and Szczesma, 2004).

The uses of bee venom in medicine are diverse, due to its analgesic, anti-inflammatory, anti-cancer, or immuno-modulatory properties. Traditionally, the use of bee venom in therapeutics is done by its direct introduction, through the sting of the bee directed to the affected area. Scientific and technological progress has made the development of other application methods possible, such as by injection, after its prior purification, or topically, locally, in the form of ointments. Numerous studies have demonstrated the effectiveness of bee venom therapy in relieving the symptoms of various diseases, such as rheumatoid arthritis, osteoarthritis, multiple bursitis, tendinitis, sclerosis, psoriasis, skin diseases, microbial infections, and even chronic fatigue (Ali, 2012; Khalil *et al.,* 2021).

The purpose of this work was to test the effects of bee venom administration by its experimental inoculation in the traditional way. Hematological investigations were carried out by determining specific blood indicators at different time intervals and at varying doses of venom. This work is important in the aspect of knowing the acute toxicity effects of the administration of bee venom to rabbits, on the erythrocyte, platelet, and leukocyte series, with the possibility of extrapolating the results obtained in human medicine studies.

MATERIAL AND METHODS

To achieve the proposed objectives, investigations were carried out on 10 rabbits divided into two equal groups of 5 animals each, taking into account the volume and frequency of bee venom administration. The 10 rabbits of the Albino and Himalayan genetic varieties were aged between four and six months, the distribution of the five females and five males into groups was as follows: two females and three males, for group 1 (G_1) and three females and two males, for group 2 (G_2). All subjects were clinically healthy at the time of their entry into the experiment, with the results of hematological determinations at time T_0 as the reference results assimilated to a control group. The principles and norms of ethics and conduct in experimental research were respected, considering the rights and protection of animals included in such experiments. The study was approved by Ethical Committee of the Faculty of Veterinary Medicine of Iași, Romania. Stings directed by bees were applied to a trimmed area of the nape, as follows: in G_1, three stings each from day 1 to day 7, after which from the seventh day the dose was reduced to a single sting/per capita/per day; in G_2, six stings from day 1 to day 7, and two stings after the 7th day. The reduction of the venom dose by decreasing the number of stings received from day 7 until the end of the administration period related to this experiment, i.e. day 14, was made conditional on the local reactivity observed after the first week of administration.

The accommodation of the rabbits was done in cages adapted to their needs for feeding, watering, bedding and excrement removal, the diet being the same for all rabbits, with water ad libitum and keeping the temperature within the thermal comfort limits, without inducing thermal stress under any of its forms, of hypo- or hyperthermia.

Blood samples were collected from the marginal auricular vein or from the saphenous vein, by the classic collection method, after performing local stasis, directly in EDTA tubes. The first collection of blood samples was carried out 3 hours after the initial inoculation of bee venom [T_1 (3h)], subsequently three other collections were carried out, two of them during the experimental period of bee venom inoculation, at

seven [T_2 (7d)] and 14 days [T_3 (14d)] respectively, from the initial administration, and a third, 7 days after stopping the inoculation from day 14, i.e. on day 21 from the initial time [T_4 (21d)], the last one determination being with the aim of observing the way of restoring the normal hematological values.

The determination of hematological parameters was done with an automatic analyzer, Abaxis VetScan HM5, including of the following parameters for analysis: *(i)* the number of red blood cells (RBC), the amount of hemoglobin (HGB), the share of red blood cells from blood – hematocrit (packed-cell volume PCV), the mean corpuscular hemoglobin MCH, mean corpuscular hemoglobin concentration (MCHC), the mean corpuscular volume (MCV), platelets (PLT); *(ii)* total white blood cell count (WBC), neutrophils (Ne), eosinophils (Eo), basophils (Ba), monocytes (Mo), and lymphocytes (Lymph). Blood smears were stained by the MGG method and examined microscopically to differentiate unsegmented (unsNe) and segmented (sNe) neutrophils.

The obtained results were statistically processed. Basic statistical indicators, such as average and standard deviation were calculated for each data population included in the study. The testing of the significant difference between the averages was done using the *One-way* ANOVA test.

RESULTS AND DISCUSSIONS

The results of the erythrocyte and platelet hematological determinations at T_0, T_1, T_2, T_3 and T_4 are presented in Table 1. The results of the tests to verify the significance of the difference between the averages are presented in Table 2.

Table 1. The evolution of erythrocyte and platelet parameters in the investigated rabbits at the time T_0, T_1, T_2, T_3, and T_4 within the experiment

Group	Time	RBC *(x 10^6 µL)*	HGB *(g/dL)*	PCV *(%)*	MCH *(pg)*	MCHC *(g/dL)*	MCV *(µm^3)*	**PLT *(x 10^3 µL)***
G_1	T_0	6.17±0.23	12.12±0.57	38.18±1.3	19.64±0.7	31.9±0.51	61.4±1.67	564.6±127.6
	T_1	5.99±0.22	11.64±0.31	36.88±0.8	19.42±0.69	31.26±0.96	61.6±1.52	519±125.1
	T_2	5.86±0.22	11.7±0.27	35.94±0.88	20±0.51	31.96±0.99	61.2±1.48	601.6±31.95
	T_3	5.86±0.3	12.12±0.48	36.16±1.28	20.68±0.59	33.1±0.8	61.8±1.92	**710.6±91.05**
	T_4	5.79±0.41	11.58±0.42	35.7±0.91	20.24±1.39	32.44±1.24	61.8±3.42	**693±89.94**
G_2	T_0	6.27±0.4	12.34±0.44	38.4±1.69	19.7±0.6	32.1±0.37	61.4±1.67	525.4±70.61
	T_1	5.97±0.18	11.6±0.26	36.52±0.92	19.44±0.38	31.3±0.81	60.8±1.48	568.6±76.84
	T_2	5.86±0.32	11.34±0.29	35.3±1.22	19.34±0.63	31.82±0.64	60.4±1.62	**835.8±100.17**
	T_3	6.15±0.49	12.34±0.71	37.98±2.16	20.08±0.59	32.5±0.98	61.8±1.79	**813.4±123.29**
	T_4	6.29±0.7	11.9±0.94	37.4±2.05	18.96±1.47	31.81±1.78	59.8±3.83	**807.6±77.86**
Reference values *(Merck, 2008)*		5-8	10-17	35-50	17-24	29-37	58-67	250-650

Table 2. The significance of differences between the means of erythrocyte and platelet parameters

Parameters	G_1 $T_0 \div T_4$ $\overline{X}\pm\sigma_x$; *(4 df – degrees of freedom)*	G_2 $T_0 \div T_4$ $\overline{Y}\pm\sigma_y$ *(4 df – degrees of freedom)*	G_1; G_2 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*				
			T_0	T_1	T_2	T_3	T_4
RBC	p=0.275; *p>0.05*; ns	p=0.506; *p>0.05*; ns	p=0.641; *p>0.05;* ns	p=0.879; *p>0.05;* ns	p=0.964; *p>0.05;* ns	p=0.292; *p>0.05;* ns	p=0.202; *p>0.05;* ns
HGB	p=0.127; *p>0.05*; ns	**p=0.049; *p<0.05*; s**	p=0.514; *p>0.05;* ns	p=0.828; *p>0.05;* ns	p=0.051; *p>0.05;* ns	p=0.581; *p>0.05;* ns	p=0.505; *p>0.05;* ns
PCV	**p=0.017; *p<0.05*; s**	p=0.057; *p>0.05*; ns	p=0.817; *p>0.05;* ns	p=0.527; *p>0.05;* ns	p=0.368; *p>0.05;* ns	p=0.144; *p>0.05;* ns	p=0.129; *p>0.05;* ns
MCH	p=0.201; *p>0.05*; ns	p=0.311; *p>0.05*; ns	p=0.888; *p>0.05;* ns	p=0.956; *p>0.05;* ns	p=0.105; *p>0.05;* ns	p=0.143; *p>0.05;* ns	p=0.266; *p>0.05;* ns
MCHC	p=0.059; *p>0.05*; ns	p=0.478; *p>0.05*; ns	p=0.5; *p>0.05;* ns	p=0.945; *p>0.05;* ns	p=0.797; *p>0.05;* ns	p=0.318; *p>0.05;* ns	p=0.535; *p>0.05;* ns
MCV	p=0.989; *p>0.05*; ns	p=0.66; *p>0.05*; ns	p=1; *p>0.05*; ns	p=0.427; *p>0.05;* ns	p=0.447; *p>0.05;* ns	p=1; *p>0.05;* ns	p=0.409; *p>0.05;* ns
PLT	**p=0.027; *p<0.05;* s**	**p=0.00009; *p<0.05*; s**	p=0.564; *p>0.05;* ns	p=0.472; *p>0.05;* ns	**p=0.001; *p<0.05; s***	p=0.172; *p>0.05;* ns	p=0.144; *p>0.05;* ns

From the analysis of the data presented in Table 1, it can be observed that the only parameter whose average values exceeded the normal range of variation, found as a reference value in the specialized literature, was the PLTs. This excess was a significant one, found for both experimental groups, at different time points after the experimental administration of bee venom. Thus, for G_1, average values greater than the maximum limit of the normal variation interval were recorded at T_3 and T_4, and for G_2 at T_2, T_3 and T_4. Regarding the other investigated parameters, all calculated average values fell within their specific reference intervals, for any of the time of blood sampling, even after repeated administrations of bee venom. On individual analysis of the investigational data, RBC, HGB, MCH, and MCHC had individual values that did not exceed or fall below the minimum reference limit. In contrast, for PCV and MCV, individual values lower than the corresponding inferior limit of the normal variation range were found.

Table 2 shows the data calculated for the *p* indicator and compared it with the standard probability value of 0.05 to test the statistical significance of the difference between the calculated averages. Significant differences between the averages were identified in the analysis of variance within each group, along the four times of blood samples harvesting, as follows: in G_1, for PCV and PLTs, and in G_2, for HGB and PLTs. In the case of HGB, such a variation has no clinical significance, considering that all individual values are within the normal limits of the reference range. In the case of PCV, the presence of each one individual of the each of the two groups with values lower than the inferior reference limit at each of the moments T_2, T_3 and T_4, can justify a wider variation within each group in part, which proved to be significant from a statistical point of view. Regarding PLTs, the determined values showed a statistically significant variability, with significant differences for each of the two investigated groups. The intergroup variance analysis revealed statistically significant differences between G_1 and G_2, at T_2, for PLTs. This moment corresponds to all the values of this hematological parameter falling within the limits of the normal range of variation, in the case of G_1, and exceeding the maximum limit, for G_2 individuals.

Table 3 shows the results of statistical significance test of differences between calculated averages for G_1, the hematological parameters that were previously confirmed with significant differences over the T_0-T_4 moments of the experimental protocol being now included in the analysis (PCV, PLT). The test of significance of difference between the averages was done for each individual moment compared to T_0. For PCV, there are no statistically significant differences between the values obtained at T_1 compared to T_0. Instead, these differences were found when testing T_0;T_2 (T_2 compared to T_0) and T_0;T_4 (T_4 compared to T_0). Although the T_0;T_3 *p*-value is statistically considered nonsignificant, it might be assumed as one which delimits nonsignificant/significant levels of testing. Surprisingly, for PLT only one variation at the nonsignificant/significant limit was found when testing the significance of the differences between the averages, and this was for T_0;T_3; statistically nonsignificant differences were confirmed for the other reported data to T_0.

Table 3. The G_1 intragroup significance of differences between the means of erythrocyte and platelet parameters of each time reported to T_0

Parameters	G_1 $T_0 \div T_4$ $\overline{X}\pm\sigma_x$; *(4 df – degrees of freedom)*	T_0; T_1 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_2 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_3 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_4 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*
PCV	**p=0.017; *p<0.05;* s**	p=0.136; *p>0.05;* ns	**p=0.017; *p<0.05;* s**	p=0.052; *p>0.05;* ns	**p=0.011; *p<0.05;* s**
PLT	**p=0.027*; p<0.05;* s**	p=0.584; *p>0.05;* ns	p=0.547; *p>0.05;* ns	p=0.071; *p>0.05;* ns	p=0.103; *p>0.05;* ns

Table 4 shows the results of statistical significance test of differences between calculated averages for G_2, the hematological parameters that were previously confirmed with significant differences over the T_0-T_4 moments of the experimental protocol being now included in the analysis (HGB, PLT). For HGB, statistically significant differences were found when T_1 and T_2 data were compared to T_0, this fact no longer being confirmed for the comparative analyzes T_0;T_3 and T_0;T_4. In the case of PLT, although no statistically significant differences was found in the analysis of the variance between T_0;T_1 values, this was confirmed throughout the other stages of the experiment, comparatively T_0;T_2, T_0;T_3 and T_0;T_4. However, both in the case of G_1 and G_2 individuals, PLT represented the only parameter whose determined values were found outside the physiological range of variation, while PCV and HGB were within the physiological normal range of variation of the investigated species.

Table 4. The G_2 intragroup significance of differences between the means of erythrocyte and platelet parameters of each time reported to T_0

Parameters	G_2 $T_0 \div T_4$ $\overline{X}\pm\sigma_x$; *(4 df – degrees of freedom)*	T_0; T_1 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_2 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_3 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_4 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*
HGB	**p=0.049; *p<0.05*; s**	**p=0.012; *p<0.05*; s**	**p=0.003; *p<0.05*; s**	p=1; *p>0.05;* ns	p=0.369; *p>0.05;* ns
PLT	**p=0.00009; *p<0.05*; s**	p=0.382; *p>0.05;* ns	**p=0.0005; *p<0.05*; s**	**p=0.002; *p<0.05*; s**	**p=0.003; *p<0.05*; s**

The presented results show a slow myelosuppressive effect on the erythrocyte series starting from the seventh day of inoculation with low doses of bee venom, but also a direct and immediate lytic effect of bee venom on red cells at 3h and in the first week of inoculation with high doses of bee venom, hemolysis being the cause of the erythroid regenerative reaction in the last 14-21 days. Our findings are similar to those reported by Yousefpoor *et al.* (2022), who demonstrated that subcutaneous injection of bee venom in healthy rats induces a decrease in RBC count, hemoglobin concentration and hematocrit, with increasing MCV and RDW (red distribution width), but without significant change in MCH and MCHC. Melittin in bee venom has been shown to have a direct lytic effect on circulating RBCs, so RBC, hemoglobin and hematocrit levels in bee venom-injected rats decreased proportionally with increasing the dose (Yousefpoor *et al.*, 2022). Some studies revealed ultrastructural reactions of bone marrow erythroid series to bee venom. Florea and Crăciun (2013) experimented with a daily administration of bee venom to Wistar rats, 700µg/kg, for 5 days and 30 days respectively, with different results considering the period of the study. Therefore, after 5 days, a reduction of bone marrow cellularity, with necrotic proerythroblasts, polymorphous erythroblasts, reticulocytes with cytoplasmic extensions and RBCs with anisocytosis and poikilocytosis (mainly acanthocytes) were reported, while, in the second case, after 30 days, the architecture of the marrow was restored, but the polymorphism of erythroblasts and reticulocytes was preserved, RBCs being more numerous but smaller changes specific to stress erythropoiesis (erythroblasts having abnormal mitochondrial cristae).

In our research, an increase in the number of blood platelets and the onset of thrombocytosis in rabbits starting from the first week of inoculation with high doses of bee venom and only from the second week of inoculation with low doses of venom, were reported. In another study (El-Abd *et al.*, 2013), platelets increased in chronic hepatitis patients treated with increasing doses of bee venom for 7 consecutive days. The increase in the number of platelets because of the injection of bee venom is explained by the activation of some cytokines, mainly IL6, which determines the maturation of bone marrow megakaryocytes, the precursors of blood platelets (Pamuk *et al.*, 2008, cited by Mohammed and Hassan, 2019).

The results of leukocyte hematological determinations at T_0, T_1, T_2, T_3, and T_4 are presented in *Table 5*. The results of the tests for verifying the significance of the difference between the averages are presented in Table 6.

Table 5. The evolution of the leukocyte parameters in the investigated rabbits at the time T_0, T_1, T_2, T_3, and T_4 within the experiment

Group	Time	**WBC (total #)**	Including (%):						
			unsNe	seNe	Eo	**Ba**	**Mo**	Lymph	Ne / Lymph
G_1	T_0	5.9±0.5	**2.475±1**	20.62±1.91	3±1.95	**9.1±1.1**	1.9±1.1	64.8±4.47	0.3±0.062
	T_1	8.52±1.1	**3.3±0.43**	31.32±3.23	2.74±0.87	**10.64±1.71**	1.24±0.57	50.43±4.99	0.63±0.13
	T_2	8.7±2.1	**1.2±0.35**	33.48±3.24	2.4±1.54	**11.2±1.53**	**6.7±2.12**	42.26±6.04	0.81±0.19
	T_3	10.84±2.17	**1.5±0.3**	26.94±6.91	3±0.21	**8.46±2.96**	2.04±0.57	58.2±7.63	0.48±0.2
	T_4	10.36±2.46	**1.2±0.46**	25.42±5.06	1.96±0.66	**7.84±2.16**	**0.94±0.67**	62.6±5.42	0.41±0.11
G_2	T_0	7.16±0.74	**1.66±0.55**	21.6±5.54	1.12±0.69	**11.48±2.58**	1.82±0.87	62.34±7.53	0.36±0.15
	T_1	7.36±0.7	**2.62±0.69**	43.96±11.54	1.62±0.7	2.62±0.8	**4.1±3.55**	44.88±11.61	1.07±0.49
	T_2	7.8±0.55	**1.82±0.24**	32.32±3.09	**5.16±1.68**	**6.98±1.29**	3.92±2.34	47.94±4.62	0.67±0.13
	T_3	9.32±0.72	**2.34±0.8**	26.04±9.2	1.92±0.28	**7.9±1.44**	1.1±0.26	60.68±10.07	0.45±0.22
	T_4	8.82±1.09	**1.7±1.23**	30.58±1.85	3.08±0.8	**7.56±1.88**	1.98±0.71	55.08±6.11	0.56±0.1
Reference values *(Merck, 2008)*		5÷12.5	0.02±0.1*	20÷75	1÷4	1÷7	1÷4	30÷85	

* Schalm's Veterinary Hematology, Sixth ed.

Table 6. The significance of differences between the means of leukocyte parameters

Parameters	G_1 $T_0 \div T_4$ $\overline{X}\pm\sigma_x$; *(4 df – degrees of freedom*	G_2 $T_0 \div T_4$ $\overline{Y}\pm\sigma_y$ *(4 df – degrees of freedom*	G_1; G_2 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*				
			T_0	T_1	T_2	T_3	T_4
WBC	**p=0.003; *p<0.05; s***	**p=0.0009; *p<0.05; s***	**p=0.014; *p<0.05; s***	p=0.081; *p>0.05*; ns	p=0.381; *p>0.05*; ns	p=0.175; *p>0.05*; ns	p=0.237; *p>0.05*; ns
unsNe	**p=0.00003; *p<0.05; s***	p=0.23; *p>0.05*; ns	p=0.355; *p>0.05*; ns	p=0.099; *p>0.05*; ns	**p=0.012; *p<0.05; s***	p=0.059; *p>0.05*; ns	p=0.419; *p>0.05*; ns
seNe	**p=0.0015; *p<0.05; s***	**p=0.0013; *p<0.05; s***	p=0.718; *p>0.05*; ns	**p=0.046; *p<0.05; s***	p=0.578; *p>0.05*; ns	p=0.865; *p>0.05*; ns	p=0.064; *p>0.05*; ns
Eo	p=0.623; *p>0.05*; ns	**p=0.00001; *p<0.05; s***	p=0.076; *p>0.05*; ns	p=0.055; *p>0.05*; ns	**p=0.027; *p<0.05; s***	**p=0.0001; *p<0.05; s***	**p=0.043; *p<0.05; s***
Ba	p=0.073; *p>0.05*; ns	**p=0.000003; *p<0.05; s***	p=0.146; *p>0.05*; ns	**p=0.00001; *p<0.05; s***	**p=0.015; *p<0.05; s***	p=0.714; *p>0.05*; ns	p=0.832; *p>0.05*; ns
Mo	**p=0.000001; *p<0.05; s***	p=0.091; *p>0.05*; ns	p=0.208; *p>0.05*; ns	p=0.113; *p>0.05*; ns	p=0.088; *p>0.05*; ns	**p=0.001; *p<0.05; s***	**p=0.043; *p<0.05; s***
Lymph	**p=0.00006; *p<0.05; s***	**p=0.013; *p<0.05; s***	p=0.976; *p>0.05*; ns	p=0.355; *p>0.05*; ns	p=0.134; *p>0.05*; ns	p=0.672; *p>0.05*; ns	p=0.074; *p>0.05*; ns
Ne / Lymph	**p=0.0004; *p<0.05; s***	**p=0.003; *p<0.05; s***	p=0.722; *p>0.05*; ns	p=0.087; *p>0.05*; ns	p=0.214; *p>0.05*; ns	p=0.849; *p>0.05*; ns	p=0.054; *p>0.05*; ns

From the analysis of the data presented in Table 5, exceeding the superior limits of the normal ranges of variation were recorded for unsNe for both experimental groups, regardless of the time of blood sampling. In the case of Ba, a situation within the range of variation was found for G_2, T_1. Average Mo values exceeded the maximum limit of the normal range of variation for G_1, T_2, and G_2, T_1. Average Eo values also exceeded the maximum limit of the normal range of variation for G_2, T_2.

As for testing the significance of the difference between the averages found for the investigated leukocyte indicators, statistically significant differences were recorded within each the group, for G_1, for WBC, unsNE and seNE, Mo, Lymph, and in the case Ne/Lymph ratio. For the WBC, the statistically confirmed variations were within the normal limits of the range of variation, as well as for the seNE and Lymph. In the case of Ba, exceeding the upper limit of the normal range of variation was without significant differences throughout the entire period of the experiment. In the case of Mo, there are significant differences between the values recorded within G_1, a fact easily observed by analyzing the average values that are significantly distinct between the different moments of laboratory sample collection. In the case G_2, the pattern of variation was different, in the sense of finding some significant differences

between the values analyzed from T_0 to T_4, for WBC, seNe, Eo, Ba, and Lymph. Among these indicators, only basophils exceeded the superior limit of the normal variation range as average values, for all time points except T_1.

The analysis of the differences between the two groups for the investigated leukocyte indicators shows significant differences for WBC at T_0, unsNE at T_2, seNe at T_1, Eo at T_2-T_4, Ba at T_1 and T_2, and Mo, at T_3 and T_4. unsNE shows average values that exceed the superior limit of the normal range of variation for both groups. Similarly, for Ba - at T_2, the difference from T_1 being one that is easily observed by comparing the average values of the two groups currently (10.64 vs 2.62). In the case of Mo, the values recorded in T_3 fall within the normal range of variation for both groups. At T_4, there are significant differences in Mo, with an average value of 0.94 for G_1 and 1.98 for G_2.

From the analysis of *Table 7*, in the case of G_1 it can be observed that the entire WBC population presented statistically significant differences between the values determined along the five moments of blood sample collection, and by comparing each moment separately to T_0. In the case of unsNe, statistically significant differences were found between T_0;T_1 while a clearly nonsignificant difference was between T_0;T_3. An interpretation of the *p*-values as one nonsignificant/significant limit can be done for T_0;T_2 and T_0;T_4 comparative analyses. These variations are important if considered that unsNe determined values are outside the physiological range of variation for this indicator. In the case of seNe, significant differences which were found between the T_0-T_4 values were confirmed for the comparative analyzes T_0;T_1 and T_0;T_2. For Mo, the values determined for G_1 along the five moments of the experiment were found outside the species-specific reference range, with statistically significant differences also confirmed in the T_0;T_1, T_0;T_2 and T_0;T_4 analyses. For Lymph, statistically significant differences were confirmed between the values determined in T_1 and T_2 when compared to T_0. The same tendency of the significance of the difference between the averages was also observed for the Ne/Lymph ratio.

Table 7. The G_1 intragroup significance of differences between the means of leukocyte parameters of each time reported to T_0

Parameters	G_1 $T_0 \div T_4$ $\overline{X}\pm\sigma_x$; *(4 df – degrees of freedom*	T_0; T_1 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_2 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_3 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_4 $\overline{X}\pm\sigma_x$; $\overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*
WBC	**p=0.003; *p<0.05; s***	**p=0.001; *p<0.05; s***	**p=0.002; *p<0.05; s***	**p=0.001; *p<0.05; s***	**p=0.004; *p<0.05; s***
unsNe	**p=0.00003; *p<0.05; s***	**p=0.047; *p<0.05; s***	p=0.077; *p>0.05*; ns	p=0.195; *p>0.05*; ns	p=0.087; *p>0.05*; ns
seNe	**p=0.0015; *p<0.05; s***	**p=0.0002; *p<0.05; s***	**p=0.00006; *p<0.05; s***	p=0.084; *p>0.05*; ns	p=0.082; *p>0.05*; ns
Mo	**p=0.000001; *p<0.05; s***	**p=0.032; *p<0.05; s***	**p=0.0058; *p<0.05; s***	p=0.282; *p>0.05*; ns	**p=0.017; *p<0.05; s***
Lymph	**p=0.00006; *p<0.05; s***	**p=0.004; *p<0.05; s***	**p=0.0003; *p<0.05; s***	p=0.339; *p>0.05*; ns	p=0.907; *p>0.05*; ns
Ne / Lymph	**p=0.0004; *p<0.05; s***	**p=0.002; *p<0.05; s***	**p=0.0006; *p<0.05; s***	p=0.146; *p>0.05*; ns	p=0.174; *p>0.05*; ns

For G_2, although the values determined for the total WBC population are not outside the reference limits of the species, the statistical significance of the differences found per total study period was confirmed when analyzing the T_0;T_3 and T_0;T_4 data (Table 8). In the case of seNe, statistically significant differences were found in the comparative analysis of T_0;T_1, T_0;T_2 and T_0;T_4 data, which fall within the reference range of the investigated species. Ba average values were outside the normal reference range, specific to the investigated species. Differences between the averages of the entire cumulative study period were tested for statistical significance, this being confirmed by the comparative analysis of each moment T_1, T_2, T_3 and T_4, to the T_0 values, considered as control group. Lymph variations, within the normal

reference range, were confirmed for T_1 and T_2 when compared to T_0. Regarding the Ne/Lymph ratio, with the exception of the comparative analysis of T_0;T_3 variations, statistically significant differences were found for T_1, T_2 and T_4 when each one was compared to T_0.

Table 8. The G_2 intragroup significance of differences between the means of leukocyte parameters of each time reported to T_0

Parameters	G_2 $T_0 \div T_4$ $\overline{Y}\pm\sigma_y$ *(4 df – degrees of freedom*	T_0; T_1 $\overline{X}\pm\sigma_x; \overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_2 $\overline{X}\pm\sigma_x; \overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_3 $\overline{X}\pm\sigma_x; \overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*	T_0; T_4 $\overline{X}\pm\sigma_x; \overline{Y}\pm\sigma_y$ *(1 df – degree of freedom)*
WBC	**p=0.0009; *p<0.05; s***	p=0.673; *p>0.05*; ns	p=0.156; *p>0.05*; ns	**p=0.002; *p<0.05; s***	**p=0.023; *p<0.05; s***
seNe	**p=0.0013; *p<0.05; s***	**p=0.005; *p<0.05; s***	**p=0.005; *p<0.05; s***	p=0.382; *p>0.05*; ns	**p=0.009; *p<0.05; s***
Eo	**p=0.00001; *p<0.05; s***	p=0.289; *p>0.05*; ns	**p=0.001; *p<0.05; s***	**p=0.044; *p<0.05; s***	**p=0.003; *p<0.05; s***
Ba	**p=0.000003; *p<0.05; s***	**p=0.00008; *p<0.05; s***	**p=0.008; *p<0.05; s***	**p=0.027; *p<0.05; s***	**p=0.025; *p<0.05; s***
Lymph	**p=0.013; *p<0.05; s***	**p=0.022; *p<0.05; s***	**p=0.007; *p<0.05; s***	p=0.775; *p>0.05*; ns	p=0.133; *p>0.05*; ns
Ne / Lymph	**p=0.003; *p<0.05; s***	**p=0.015; *p<0.05; s***	**p=0.007; *p<0.05; s***	p=0.427; *p>0.05*; ns	**p=0.033; *p<0.05; s***

Leukocytes are effectors of the specific and non-specific immune system and increased throughout the experiment, with statistically significant differences in the G_1 experimental group starting at time T_1, and in the G_2 experimental group starting at time T_3. A non-specific neutrophilic inflammatory response occurred after inoculation of low and high doses of bee venom, with a significant increase in the Ne/Lymph ratio, more pronounced at time T_1 and T_2 compared to time T_0 in both experimental groups. The change in Ne/Lymph ratio is very evident, with a tendency to increase above 1.00 in G_2 at time T_1. Another sign of the onset of acute inflammation is the significant increase at T_1 of Ne in both groups, followed by oscillating changes during the experiment. Yousefpoor *et al.* (2022) also demonstrated that subcutaneous injection of bee venom in healthy rats induces an increase in WBC and neutrophils. In an another study carried out on human subjects - 133 children with bee stings (Etim *et al.*, 2020), hematological parameters showed significant changes only regarding leukocytes, noting an increase in the average value of WBC (28.9±1.4 X103/µL), three times compared to the WBC mean of the control group (8.9±1.6 X103/µL), as well as an increase in the number of neutrophils (Ne 23.9±2.7 X103/µl in the bee sting group, compared to 3.5±2.9 X103/µL in the control group). Leukocytosis and neutrophilia are due to the evolution of a local inflammatory process generated by the sting.

In our investigation, monocytes also showed oscillating changes, being indicators of activation of the monocyte-macrophage system, dependent on the dose of bee venom inoculated. Thus, as early as T_1, monocytes increased in response to high doses of venom, while low doses initiated the monocytic reaction at T_2. Generally, there are two macrophage invasions into the inflamed tissue, separated by a neutrophile invasion: tissue macrophages for the first line of defense, neutrophils for the second line of defense and macrophages again for the third line of defense. Finally, the fourth line of defense greatly increased production of both granulocytes and monocytes by the bone marrow (Ahmed and Schurig, 2012).

Basophils as hypersensitivity effectors had values above the upper reference limit at T_0 in both groups, which explains an already existing allergic field. However, in the G_2 group inoculated with high doses of venom, statistically significant changes were found for basophils and eosinophils. Thus, basophils were considerably reduced at T_1, rapidly passing into circulation to initiate the allergic-type inflammatory response, but gradually recovered at T_2, T_3 and T_4. In contrast, eosinophils increased throughout the experiment, but within physiological limits, with a moment of eosinophilia occurring at T_2. It is well known that the tissue mast cells and basophils release an eosinophil chemotactic factor – histamine that causes eosinophils to

migrate toward the inflamed allergic tissue. It is also known that basophils and mast cells have receptors on their cell membranes for IgE antibodies associated with allergies. As a result, basophils enhance allergic reactions, whereas eosinophils tend to dampen them (Ahmed and Schurig, 2012).

There are other studies, however, that demonstrate that the s.c administration of bee venom in animal models in which arthritis was experimentally induced (Mohammed and Hassan 2019; Salem *et al.,* 2022) had an immunosuppressive and anti-inflammatory effect, revealed by the reduction of WBC and neutrophils, due to the inhibition of some pro-inflammatory cytokines. El-Abd *et al.,* (2013) revealed a distinctly significant increase in WBC and lymphocyte counts *($p<0.01$),* but within normal limits, in patients with chronic hepatitis, after treatment with increasing doses of bee venom administered for 7 consecutive days. The increase in lymphocytes is due to the stimulation of the immune system by some compounds of bee venom, such as phospholipase A_2, the influence of bee venom in the initial activation of T lymphocyte populations and later of B lymphocyte populations and monocytes being clearly specified in the specialized literature; also, the shift from the expression of cytokines from the Th2 system to the expression of cytokines from the Th1 system, is responsible for the production of Ig G and the decrease in the production of Ig E, which explains the absence of a systemic hypersensitivity response in G_1 in the current study and in the other studies, even though bee venom contains highly allergenic compounds and immunogens (PLA2, melittin, acid phosphatase) (Hossen *et al.,* 2016).

CONCLUSIONS

The study of erythrocyte, platelet, and leukocyte parameters in rabbits, following the administration of variable doses of bee venom to individuals made up of two groups, with an approximately uniform distribution in terms of age and sex, revealed the following aspects:

The toxic action of bee venom is dependent on the dose and the daily cumulative effect, in the case of erythrocyte and platelet parameters. During the 14 days of administration, to which another 7 days of post-administration observation were associated, the bee venom progressively generated the onset of thrombocytosis and a short-term reduced hemolysis.

In the case of leukocyte parameters, the administration of bee venom caused an acute inflammatory reaction, with increasing Ne/Lymph ratio and a peak of monocytosis at low doses of venom, but also oscillating changes in unNe and basophils with an eosinophilia peak, an indicator of allergic reaction, at high doses of venom.

Authors' contribution: Geta *Pavel* (Assoc.Prof.) – Conception and coordination of the experimental framework, clinical examination and hematological analyses, para clinical interpretation of the obtained results. Dănuț *Bratu* (DVM) and Daniel *Bejenariu* (DVM) - execution of the experimental framework, bibliographic documentation, centralization of the obtained data. Andrei C. *Grădinaru* (Assoc.Prof.) - final writing of the scientific article based on the provided investigation data, statistical processing and interpretation, additional documentation.

REFERENCES

AHMED, S.A.; SCHURIG, G.G. The immune system. In: KLEIN B.G. (Ed.). *Cunningham's textbook of veterinary physiology*. 5.ed. Canada: Saunders, 2012.

ALI, M.A.A.S.M. Studies on bee venom and its medical uses. *Int. J. Adv. Res. Technol.*, v.1, p.1-15, 2012.

EL-ABD, S.; ELFIKY, A.A.; MASHHOOR, E.E.A. Study of the immunological effect of bee venom on chronic diseases in human. *Benha Vet. Med. J.*, v.25, p.183-191, 2013.

EL-WAHED, A.A.A.; KJALIFA, S.A.M.; SHEIKN, B.Y. *et al.* Bee venom composition: from chemistry to biological activity. In: RAHMAN A.U. (Eds.). *Studies in natural products chemistry*, 2019. v.60, chap.13, p.459-484.

ETIM, E.A.; COLLINS, A.O.; NWEKE, J.N. Hematological parameters of children with bee sting envenomation in Yola Northeastern Nigeria. *World J. Adv. Res. Rev.*, v.7, p.213-217, 2020.

FLOREA, A.; CRĂCIUN, C. Bee venom induced *in vivo* ultrastructural reactions of cells involved in the bone marrow erythropoiesis and of circulating red blood cells. *Micros. Microanal.*, v.19, p.393-405, 2013.

HOSSEN, M.S.; SHAPLA, U.M.; GAN, S.H.; KHALIL, M.I. Impact of bee venom enzymes on diseases and immune responses. *Molecules*, v.22, p.25, 2016.

KHALIL, A.; ELESAWY, B.H.; ALI T.M.; AHMED, O.M., Bee venom: from venom to drug. *Molecules*, v.26, p.4941, 2021.

MERCK veterinary manual. Whitehouse Station, NY: Merck & Co., Inc., 2008.

MOHAMMED, Z.I.; HASSAN, A.J. Effect of bee venom on some blood and biochemical parameters in formaldehyde induced arthritis male rats in comparison with prednisolone drug. *J. Physics: Conf. Ser.*, v.1234, p.12066, 2019.

PAK, S.C. Chemical composition of bee venom. In: ALVAREZ-SUAREZ J. (Ed.). *Bee products* – chemical and biological properties. Cham: Springer, 2017. p.279-285.

PAMUK, G.E.; VURAL, O.; TURGUT, B.; DEMIR, M., PAMUK, Ö.; ÇAKIR, N. Increased platelet activation markers in rheumatoid arthritis: Are they related with subclinical atherosclerosis? *Platelets*, v.19, p.146-154, 2008.

RYBAK-CHMIELEWSKA, H; SZCZESNA, T. HPLC study of chemical composition of honeybee (*Apis mellifera* L.) venom. *J. Apicul. Sci.*, v.48, p.103-109, 2004.

SALEM, H.S.S.; MEGAHED, H.M.; SARHAN, M.M. Bee venom for the treatment of rabbit arthritis caused by *Staphylococcus aureus. Adv. Anim. Vet. Sci.*, v.10, p.2004-2012, 2022.

YOUSEFPOOR, Y.; OSANLOO, M., MIRZAEI-PARSA, M.J. *et al*. Subcutaneous injection of bee venom in Wistar rats: effects on blood cells and biochemical parameters. *J. Pharmacopuncture*, v.25, p.250-257, 2022.