S U M M A R Y

The thesis entitled "Dynamics of some blood biochemical indices in mammals in the case of bee venom intoxication" is based on an original study conducted on lab animals' batches injected with various doses of bee venom, studying the changes produced in the main organs and tissues of the animal body, creating the extrapolation premises of these results for future therapies of veterinary and human medicine.

The thesis comprises a total of 247 pages and it is divided into two parts:

Part I - The current state of knowledge that includes a total of 83 pages, consists of three chapters containing general considerations about bee venom from a biochemical and toxicological point of view. In this part we used data from literature, few data about blood biochemical parameters in mammals and their pathophysiological implications and also the bee venom toxicity in mammals, with the presence of different pathophysiological mechanisms.

Part II - representing our own research, is the personal section also divided into three chapters, including the purpose of the thesis with the presentation materials and research methods, a chapter about the results and discussions following successive inoculations of doses in animals involved in the study and a final chapter, which contains the general conclusions of the researches

The data presented in this paper are supported by a total of 47 tables and 117 figures, graphics plus hystological images inserted in Chapter II, representing true evidence of the originality of this paper.

The bibliography at the end of this work comprises 159 titles of Romanian and foreign literature, and also electronic pages of various magazines and papers that refer to the theme.

The bibliography also includes specific legislation in the field of toxicology, standards of working methods and titles of some personal works presented in various symposia of the Faculty of Zootechnics and Veterinary Medicine, one of the works being awarded with the 1st prize in 2011.

The originality of the thesis stands in revealing the toxicity effect of bee venom on the mammal's body depending on the dose.

The numerous studies conducted so far show that bee venom has a complex structure, containing organic and inorganic substances, which give it specific, particular properties.

Bee venom is a poison secreted by the glands of worker bees and eliminated by the action of some external stimuli. Upon eliminating from the defense apparatus gall of the bee, the venom is presented as a colorless, dense liquid.

In contact with the air it quickly solidifies and its volatile components are released. By scraping the crystallized bee venom from the collection plate, this becomes a white powder - slightly gray. Bee venom is heat resistant and low temperature or high temperature does not destroy its properties. Under dry form it can be stored for a long time without losing its toxic properties. It is resistant to the action of acids and bases.

Due to the complex chemical composition, the bee venom clearly has a well defined biological action, becoming an important source of active substances for apitherapeutic products. Bee venom contains more than 40 active substances, many having biological effects. The most relevant is an anti-inflammatory compound melittine. This substance helps to produce the hormone cortisol. Since this is an anti-inflammatory, comparative studies have shown that melittine is 100 times more stronger than hydrocortisone. This is why bee venom may be necessary in treating inflammatory diseases such as rheumatoid arthritis.

Other compounds that may have pharmacological effects include apamin, which has the effect of strengthening the network of nerve pathways; adolapin, an anti-inflammatory and analgesic, norepinephrine, dopamine and seratonin, efficient in depression.

Beekeepers, who use the full range of bee products are those with the highest life span (after the category of professional orchestra conductors) and are rarely reported with cases of cancer; it is the busier socio-professional category, full of energy and health.

In the literature it is estimated that among the underlined compounds so far in bee venom (VA), the majority of them are represented by histamine, catecholamine, polyamines, apamin, melitinină, phospholipase .

Bee venom contains 46.36% carbon, 7.56% hydrogen and 13.30% nitrogen and is composed of proteins, enzymes, hormones, minerals, essential oils and other volatile substances. The main component of bee venom is protein substances in whose structure three fractions are determined. The most important fraction is the melittine protein, which is considered the most active component of venom. In melittine, one has identified glycocoll, alanine, valine, leucine, isoleucine, serine, tyrosine, lysine, arginine, asparagine, glutamine, tryptophan, proline.

This fraction of the venom is hemolytic, reduces blood pressure and the respiratory rate and blocks peripheral and central nerve gaps. It has an alkaline reaction, with pH of 11.0. Another fraction that is well represented is the one composed from phospholipase A yeast in phospholipase which has a pH 10.

The complex nature of venom must be attributed to the great diversity of predatory insects and vertebrates that can attack the bee family. In humans, the reactions to the venom drop are of three types: local, systemic and anaphylactic. In the first case the reaction, local swelling extends for several hours and the punctured place may be red, warm and sensitive for 2-3 days. A systemic reaction occurs within minutes after the sting and can cause a general rash, respiratory problems, nausea, vomiting, abdominal pain and syncopes.

In anaphylactic reaction, the symptoms are manifested in a few seconds after the sting and involve breathing difficulties, mental confusion, vomiting, shock that can lead to unconsciousness and death by respiratory and circulatory collapse. Generally, it can create some resistance to bee stings, but still their reactions can occur unexpectedly, by a cause or another, very intense. Those who are sensitive can die from a single bee sting, but a man has suffered 2243 stings and survived.

From a toxicological point of view, bee venom has a direct action - cellular toxicity (leading to the cell destruction) and an indirect action - immunological manifestations. This is due to chemical composition, which is very complex and contains enzymes, peptides, amines, amino acids and other substances that may be allergens for the organism.

From the physiological point of view all actions of bee venom are toxic. The toxicity of bee venom is given by the large poison gland of the bee. Following the sting, swelling is due to the histamine in the venom. There is no vaccine against bee venom. Some researchers believe that the vaccine antidote is the bee propolis aqueous solution, stabilized and standardized - Propolis H4. Beside its intentional and medical supervised use, bee venom should be avoided.

Bee venom action in the organism reaches the nervous system first of all. The following three actions of bee venom were described:

1. haemolytic (produces the destruction of red blood cells);
2. bleeding (produces blood output from the vessel);
3. neurotoxic.

It has been shown that the bee venom has anti-inflammatory properties. The therapeutic action of the venom is due to the melittine content, a substance with anti-inflammatory properties. Melittine stimulates body's secretion of an inflammatory hormone,

called cortisol. At the same time, melittine has antibacterial properties, stimulates production of antibodies and the system's immunity, improves microcirculation.

The purpose of the thesis is to follow the development of some blood biochemical and hematological constants in rats and rabbits inoculated with bee venom. The experiment represents the means of establishing a correlation between bee venom poisoning and blood biochemical constants change in mammals. Research is performed before clinical trials and it is based almost entirely on theory and experiments on animals.

Values of blood biochemical evolution of some mammals' species (rats and rabbits) may reflect the tendency of these constants in bee venom poisoning for all mammals.

This experimental model presented in this doctoral thesis, represents the means to determine the correlation between bee venom poisoning and the blood biochemical constants change, which is the particular working hypothesis for this experiment.

In addition to the toxic, inflammatory, allergic and sometimes even fatal effect from a single bee sting, venom has a therapeutic effect. Currently, alternative medicine has gained popularity, including bee venom therapy, due to numerous research reports, which have shown its beneficial effects.

These controversial properties attributed to the bee venom (anaphylaxis, angioedema, cardiotoxic, nephrotoxic, hemolytic and immunomodulatory, antimutagenic, antitumor, anti-inflammatory and antinociceptive effects) led us to the initiation of this study.

Consistent with its purpose, we are following the dynamics of the changes in biochemical blood indicators to variable sub-lethal doses of bee venom and response variation of the mammalian body to bee venom, depending on dose and action time.

The evolution of hematological values is also interesting, performing a complete blood count in each collection.

In the **rats** inoculated with bee venom in different doses (0.3; 0.6; 1.2 mg) we have determined the following:

- statistically significant increase in the number of red blood cells proportionally with the inoculated dose from 6.22 to 8.60 x 10^6/µl after 6 hours.

The haemoglobin increases from 10.26 g/dl to 13.75 g/dl after 9 h.

- simultaneous increase of leucocytes proportionally with the inoculated dose. In the first 6 hours from inoculation the leucocytes nearly doubles, from 3.5 to 6.5 x 10^3/µl.
- the total proteins have evolved synchronous with granulocytes.

From the study of dynamics of most parameters studied (erythrocytes, CHEM, creatinine, total proteins, GOT, GPT), the existence of two "sub-phases" of physiological evolution is determined:

- **hemoconcentration** subphase;
- **hemolitic** subphase**.**

Thus, red blood cells, creatinine, total proteins, GOT and GPT values show a progressive increase in the first 6 hours, followed by a steady decline after that time. CHEM decreases in the first sub-phase, then returns gradually to normal values. Albumin, urea, alkaline phosphatase and amylase show a constant increase up to 24 hours after the initial inoculation.

The protein dynamics in rabbits inoculated with various doses of bee venom and with periodical blood letting shows a gradual decreasing of the blood proteins in the first 6 hours after inoculation in all groups (the biggest decrease being in E1 group from 6.90 g/dl la 5.70 g/dl) followed by a gradual rebound.

Concerning the alkaline phosphatase (ALP) there is a progressive increasing from 210.5 to 504.6 iu/l after 9 hours from the inoculation with 0.1 mg bee venom.

The evolution of the serum transaminases: there are significant differences and a constant increasing of the serum level of the ALT and AST, creatinine and urea followed by a bias of decreasing after 9 hours.

Creatinine, total blood proteins, AST and ALT draw a progressive increasing of the values in the first 6 hours, folloed by a constant decreasing after this point. MCHC decreases during the first sub-phase to rebound progressively. Albumine, urea, alkaline phosphatase and amilase follow an increasing bias till 24 hours from the inoculation.

Analysing the cholesterole evolution it is observable a decreasing of the values until 6 hours (from 156.2 to 95.4 mg/dl in E1 group) folowed by an emphatic rebound.

The glycemia variations in all groups are in the physiological reference limits while the variations of the lipid metabolism values are very floating.

The lipoproteins HDL, LDL, VLDL and the chylomicrons are modified of 2 plasmatical enzymes: lecitine-cholesterole acyltransferase, with phospholipasic A2 activity and lipoproteinelipasis. It is demonstrated that phospholipases A2 from the venoms have an enzymatical activity 3 times larger than plasmatic lecitine-cholesterole acyltransferase.

Creatinine mentained in normal limits and variated unsignificantly in E2 group, while in E3 group was noticed a decreasing under the inferior reference level after 6 hours from inoculation, in 24 hours a rebound being observed.

Urea increased slowly at T1 and T2 considering T0, the values exceeding the upper reference limit, after 24 hours being observable a significant attenuation till the reference values limits.

Regarding the rabbits involved in the study, inoculated with the same venom doses, I have determined the modification of the following biological parameters:

- Bee venom administration in rabbits showed increased glucose (without exceeding the reference limit of the species) at doses of 0.3 mg per animal, starting 6 hours after inoculation, from 6.90 g/dl la 5.70 g/dl at the E1 group while at doses of 0.6 mg and 1.2 mg per animal, the increase occurred at 2 hours after inoculation, with a return to the initial values after 24 hours. Slight and transient increase of glycemia is due to the adrenergic and corticosteroid effect induced by the inoculated substance.

- The phospholipase A2 from bee venom in rabbits involved in the study has determined a significant reduction in cholesterolemy and triglyceridemia, relevant to 2 and 6 hours after inoculation, with a return to baseline by 24 hours. The effect is the result of the enzyme action in conjunction with plasma lipoproteins and the acquisition of triglycerides in the adipose tissue.

This favorable speculated body deserves to harness the therapeutic effect of bee venom to treat dyslipidemia.

The inoculation of 0.3 mg per individual bee venom in the rabbits involved in the study showed a gradual increase of the serum level of the transaminases (ALT and AST) and of urea, thus demonstrating the insidious, slow installation of the state of hepatotoxicity and renal dysfunction, even at small doses of venom.

The inoculation of 0.6 and 1.2 mg doses of bee venom per rabbit indicated an immediate increase to 2-6 hours of serum transaminases (AST and ALT) with recovery after 24 hours, but also the PAL increase to 24 hours after inoculation, changes which indicate the installing of some reversible hepatic-cellular lesions (it remains to be clarified whether the increased PAL is the colangiocellular lesion or secondary to adrenal stimulation).

The rapid increase of the urea level at doses of 0.6 and 1.2 mg of venom per rabbit, then the return to normal values after 24 hours, shows the acute renal dysfunction caused by transient reduced renal blood flow, followed by triggering some compensatory mechanisms for the restoration of homeostasis at the cellular level.

Morfopathological aspects in rats after 9 hours from the toxic inoculation:

In liver we observed: liner portal space with vascular congestion and bile ducts dilatation; lymphocites and red bloodcells in sinusoidal capillaries; hepatocytes nucleuses

with condensed chromatine; binucleation; stasis in centrilobular veins and perivascular spaces; sinusoidal microthrombosis; hepatocitolisis in central lobular area; stasis in the portal venula; hydropic degenerescence in hepatocytes; Kupfferian hypertrophy.

In myocardium we noticed: adherent red bloodcells on the endothelium; thrombosis; interstitial hemoragy; myocitolysis; interstitial lymphocitosis; distrophic lesions.

Kidneys showed: glomerular capillary congestion; glomerular hypertrophy with Bowman capsule limitation; vascular microthrombosis; interstitial hemoragy; tubular necrosis; hyalin cylinders; arterial stasis with hypertrophy of the medial layer of the vascular wall.

Striated muscle tissue showed: myocitolysis; interstitial inflamation; interstitial hemoragy.

In the spleen were observed: hyperplasia of the white pulp; vascular thrombosis; dilatated sinusoids; sinusoidal lympocitosis.

The lungs presented: thrombosis; interstitial monocytary inflamatory reaction; vascular stasis and congestion.

The hystological lesions observed in our study accord with the results of the biochemical analyses certifying the very complex toxic action of the bee venom in acute experiment, marked by circulatory, inflammatory and degenerative disorders in liver, kidney, lungs and myocardium. These do not exclude the therapeutical action of the venom but suggest caution in the clinical evaluation of the patients, because the pro- and antiinflammatory actions could be used in medical prophylactic and therapeutical purposes.