

STUDIES ON MODIFICATIONS OF THE HEPATIC AND RENAL BIOCHEMICAL PARAMETERS OF RABBITS AFTER BEE VENOM INOCULATION

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Abstract

The experimental study reported in the present paper was performed on rabbits for making evident the effect of various bee venom doses on dynamics of exploration serum biochemical parameters of hepatic and renal function. On this purpose three experimental groups were chosen and bee venom inoculated by three stings (E1 group), 6 stings (E2 group) and 12 stings (E3 group). Prior to inoculation and at time intervals of 2 h, 6 h and 24 h after inoculation blood samples were taken off and submitted to biochemical investigations (ALT, AST, GGT, alkaline phosphatase - ALP, creatinine and urea).

The obtained results made evident a hepatic cytotoxic effect (by ALT and AST increase) noticed 24 h. after inoculation at the E1 group and even after 2-6 h. at the E2 and E3. groups. The serum level of ALP was found to decrease slightly 2 and 6 h. after inoculation for every experimental group while after 24 h a clear increase although below the complete limit of the reference values was noticed with the E2 and E3 groups only. The serum concentration of GGT was found to decrease gradually along the 24 h. interval for every group under study although between physiological limits. As regards the renal biochemical parameters a surprising evolution was noticed - at the E3 group the creatinine decreased below the minimum reference limit 6 h. after inoculation and the urea already increased in the inoculation time was continuously increasing 2h and 6h after inoculation, decreasing to the normal value limits after 24 h at E2 and E3.

In conclusion, the present study made evident an acute and subacute hepatotoxic effect of the bee venom depending on the dose but did not confirm the nephrotoxic effect sustaining on the contrary the theory of the beneficial effect for the homeostasis of the organism metabolic processes.

Key words: bee venom, sting, rabbit, ALT, AST, GGT, ALP, creatinine, urea

INTRODUCTION

The liver and the kidney are target organs for the lesions occurred by the organism exposing to aggressions caused by polluting chemicals, drugs or natural toxic agents (toxic plants, reptile and arthropod venom [5, 8, 9]).

The present study is aimed to investigate the effect of the bee venom on some serum indicators of the hepatocellular (transaminases) and colangiocellulare (alkaline fosfatase) functions, as well as of the glomerular and renal tubular function (creatinine, urea nitrogen), in function of the applied venom dose and of the evolution study of the generated process.

The bee venom is a complex mixture of proteins, small peptides, enzymes, minerals

and acids among which the melitin, the fosfolipase A2, the degranulation peptide of mastocytes and apamin show the highest toxic, allergic and inflammatory potential. Although the pathology of the stings of venomous insects describes the anaphylaxy, the angioneurotic edema and the lethal effect caused by only one bee sting as well as the cardiotoxic, hepatotoxic, nephrotoxic and homolytic effects dependent on the dose the alternative medicine is nowadays of a continuously increasing interest the therapy with bee venom being include. Numerous scientific reports made evident the immunomodulation, antimutagenic, antitumoural, antiinflammatory and antinociceptive effects of this therapy [5, 9].

These controversial properties of the bee venom have initiated the present study to settle the modification dynamics of the blood biochemical indicators when a variable non-lethal dose of bee venom is administered.

MATERIALS AND METHOD

A number of 18 rabbits of 6-7 months old and weighting 0,700-0,900 kg, were taken in the study and maintained under identical feeding and care conditions. They were divided into three groups of 6 rabbits each and directed bee stings were then applied in their auricular region.: E1 group - 3 stings per animal, E2 group - 6 stings per animal, E3 group-12 stings per animal (one sting is equivalent to 0.1 mg of venom). Before inoculation and then 2, 6 and 24 h., respectively, after inoculation blood samples were collected into dry tubes and the serum for the determination of the hepato-renal biochemical parameters obtained (ALT – alanin aminotransferase, AST – aspartat aminotransferase, GGT – gamma-glutamyltransferase ALP – alkaline phosphatase, serum urea, serum creatinine), by using the automatic biochemical analyzer Accent 200 (Faculty of Veterinary Medicine Iași).

RESULTS AND DISCUSSION

The obtained results are presented in Table 1 and the significant differences are made evident in graphs in fig. 1-4.

With the E1 group (3 bee stings applied equivalent to 0.3 mg venom), un non-significant increase in the serum level of ALT 6 h after inoculation .is noticed (143.6 ± 15.90 UI/l), the increase becoming significant 24 h. after inoculation (190.3 ± 10.35 UI/l). The average value of ALT at the time T0 was above the reference upper limit (124.7 ± 23.25 UI/l, towards 80 UI/l). AST increased gradually from 43.4 ± 2.20 UI/l at the time T0 to 147.8 ± 7.60 UI/l at the time T3, exceeding the reference upper limit of the species. ALP (alkaline fosfatase) and GGT decreased at T1 (2 h. after inoculation),

coming then back gradually at T2 and T3 to the initial values. These variations were situated between the known reference limits Among the investigation parameters of the renal function, the blood urea showed an increase at T2 and T3 with no entire coming back to normal values 24 h. after inoculation. Again an increased value against the reference upper limit at the time T0 is noticed.

The modification dynamics of the biochemical parameters of hepatic function exploration showed almost an identical evolution for the groups E2 (6 bee stings equivalent to 0.6 mg of venom) and E3 (12 bee stings, equivalent to 1.2 mg of venom). The serum level of ALT showed a progressive increase at T1 and T2 and was maintained also at T3, while the AST level increased at a maximum at T2 (2-4 fold in comparison with T0 and above the upper reference limit of the species) with the tendency of attaining the normal limits but not the initial value at T3 . ALP showed a slight decrease at T1 and T2 in comparison with T0 followed by a clear increase 24 h after inoculation, proportional to the amount of inoculated venom. The creatinine remained between normal limits and varied non-significantly at the group E2, while with the E3 group it decreased below the reference minimum limit 6h. after inoculation returning then to the minimum limit 24 h. later. The urea increased slightly at T1 and T2 in comparison with T0, these values exceeding the reference upper limits decreasing then significantly to the reference limit values 24 h

The obtained results reported in the present paper made evident the fact the bee venom dose of 0.3 mg per rabbit resulted in a progressive increase in the serum transaminases which would be indicative of an insidious and slow hepatocellular, while the doses of 0.6 and 1.2 mg per rabbit cause a sudden increase of the serum transaminases 2 h after inoculation followed by a tendency of returning to the initial values 24 h. after inoculation (fig. 1, 2).

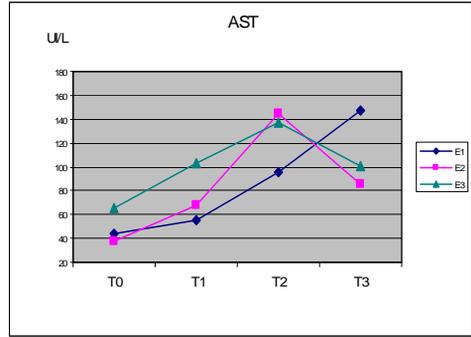
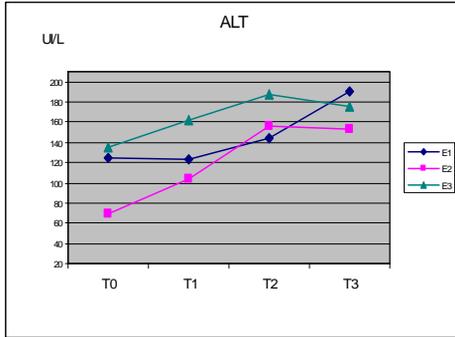


Fig.1. Variation of the serum level of ALT at rabbits inoculated with bee venom in function of dose and the moment of sample drawing (group E1 - 0.3 mg individual dose; group E2 - 0.6 mg individual dose; group E3 - 1.2 mg individual dose; T0 - before inoculation; T1 - 2h after inoculation; T2 - 6h. after inoculation; T3 - 24h after inoculation).

Fig.2. Variation of the serum level of AST at rabbits inoculated with bee venom in function of dose and the moment of sample drawing (group E1 - 0.3 mg individual dose; group E2 - 0.6 mg individual dose; group E3 - 1.2 mg individual dose; T0 - before inoculation; T1 - 2h after inoculation; T2 - 6h. after inoculation; T3 - 24h after inoculation).

Table 1. Dynamics of blood biochemical parameters of hepatic and renal function exploration (average values and standard deviation) at rabbits inoculated with various doses of bee venom (E1 group-3 stings, E2 group-6 stings, E3 group -12 stings; M - control group).

Group	Time of sample drawing	Hepatic biochemical parameters				Renal biochemical parameters	
		ALT ui/l	AST ui/l	ALP ui/l	GGT ui/l	Creatinine mg/dl	Urea mg/dl
E1 (n = 6)	T0	124.7 ± 23.25	43.4 ± 2.20	122.5 ± 18.35	4.25 ± 0.05	1.19 ± 0.07	39.27 ±2.00
	T1	123.1 ± 19.85	55.25 ± 6.75	99.9 ±13.10	2.1 ±0.55	1.07 ±0.21	43.90 ± 8.11
	T2	143.6 ±15.90	95.8 ±52	109.9 ±21.30	2.3 ±0.20	1.05 ±0.05	49.86 ±4.71
	T3	190.3 ±10.35	147.8 ±7.60	115.3 ±5.15	4.1 ±0.06	1.14 ±0.02	45.9 ±0.85
E2 (n = 6)	T0	69.1 ± 16.00	37.9 ± 4.20	129.65 ± 46.85	5.05 ± 2.75	1.10 ± 0.15	43.84 ± 0.96
	T1	104.5 ± 6.60	67.6 ±8.85	124.6 ±55.0	4.7 ±1.40	1.1 ±0.15	48.74 13.53±
	T2	156.2 ±7.25	144.9 ±20.45	124.6 ±46.70	4.7 ±1.45	0.8 ±0.20	47.08 ±9.61
	T3	152.7 ±20.3	85.4 ±14.4	174.85 ±35.45	3.65 ±0.65	1.0 ± 0.2	28.16 ±0.28
E3 (n = 6)	T0	135.1 ± 4.20	65.6 ± 18.40	129 ± 16.40	5.2 ± 0.90	1.13 ± 0.06	32.97 ± 0.90
	T1	161.9 ±5.65	103.2 ±23.30	117.4 ±11.70	4.1 ±1.65	1.12 ±0.06	39.84 ±1.20
	T2	187.5 ±23.35	137.4 ±33.20	124.2 ±13.20	3.6 ±0.90	0.05 ±0.01	42.85 ±1.49
	T3	175.7 ±23.55	100.1 ±18.85	224.1 ±93.45	2.9 ±1.45	0.5 ±0.30	28.7 ±0.00
M (n = 18)		109.6	48.96	127,0	4.83	1.14	38.69
Reference values (Merck, 2008)		48-80	14-133	61-283	0-14	0.5-2.5	13 -29

T0-before inoculation; T1-2 hours after inoculation; T2-6 hours after inoculation; T3-24 hours after inoculation.

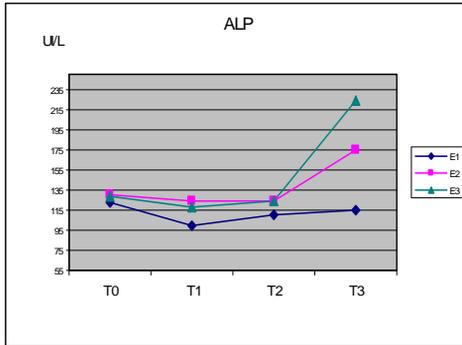


Fig. 3. Variation of the serum level of ALP at rabbits inoculated with bee venom in function of dose and the moment of sample drawing (group E1 - 0.3 mg individual dose; group E2 - 0.6 mg individual dose; group E3 - 1.2 mg individual dose; T0 - before inoculation; T1 - 2h after inoculation; T2 - 6h. after inoculation; T3-24h after inoculation).

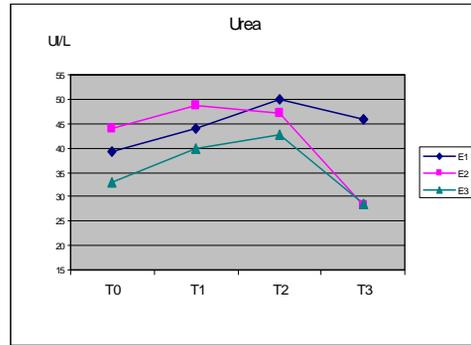


Fig.4. Variation of the serum level of urea at rabbits inoculated with bee venom in function of dose and the moment of sample drawing (group E1 - 0.3 mg individual dose; group E2 - 0.6 mg individual dose; group E3 - 1.2 mg individual dose; T0 - before inoculation; T1 - 2h after inoculation; T2-6h. after inoculation; T3 - 24h after inoculation).

These modifications are correlated with the occurrence of some reversible hepatocellular lesions. In addition to that, with higher doses an increase in the serum level of ALP is noticed although 24 h. after inoculation only (fig. 3) which is also relevant for the occurrence of colangiocellular or corticosuprarenal lesions.

The increase in the serum urea 2 and 6 h. after the bee venom inoculation with all the three experimental groups followed by coming back to normal values after 24 h. (fig.4) are indicative of a quick renal damaging due to the modification of the renal hemodynamics as well as of the starting of the compensatory mechanisms for the renal function recovery. Our results are supported by data to be found in scientific literature, the toxic effects of melittine as the main poison in the bee venom being proved [6, 9]. Thus, A. Florea et al. found that low doses of bee venom cause some reversible hepatic lesions to rats along with the starting of the defense mechanisms and cellular detoxification while high doses cause much more serious and irreversible alterations by hepatocellular necrosis and disseminated intravascular coagulation [2].

Other studies maintain that the small peptides in the bee venom cause the inhibition of the activity of the metabolic

enzymes such as ALP, AST, ALT, LDH, AChE. The most of the researchers revealed the increase of the serum enzyme levels due to the muscle, hepatic or renal necrosis and to the release of the enzymes into the blood. (R.K. Upadhyay et. al., 2010).

Hassanein and Hegab, 2010, have demonstrated that the rats exposed simultaneously to the intoxication with lead acetate and low doses of bee venom, show an improvement of the hepatic toxicity while higher bee venom doses result in the intensification of the toxic effect revealed by the increase in the nitrate and nitrite levels in the hepatic tissue as well as by the increase of the indicators of the hepatic cell oxidative stress (superoxide dismutase, glutathione, malondialdehyde) [4, 5].

Betten et al., 2006, made evident numerous systemic effects given by the bee stings and inoculation of high venom amounts to human beings. Due to the cytotoxic effect of the bee venom the hemolysis, hemoglobinuria, rhabdomyolysis and the transient increase of the serum transaminases caused by hepatic and muscular cytolysis have occurred after 24 h.

Neuman M.G., 1991, has induced the hepatotoxicity to dogs and cats by inoculating various doses of wasp venom sack extract and found an increase of the

serum transaminases, ALP and also of β -N-acetyl hexozaminidase (BNAH) as a function indicator of the Kupffer cells [7].

In order to settle the mechanisms of the acute renal insufficiency induced by the bee venom Grisotto et al., 2006, evaluated the renal function of rats after injecting bee venom (0.5 mg/kg) and found a temporary decrease of the glomerular filtration rate and of the renal blood flux as well as a significant increase of the serum enzymes (CPK, LDH, GPT) after 70 min. which proves the presence of rhabdomyolysis. The renal blood flux was recovered after 24 h but the renal dysfunction still persisted with lesions of tubular necrosis being made evident in the zone of the contort proximal tube [3].

Many other authors pointed out the same causes of the renal insufficiency induced by the bee venom: renal vasoconstriction, direct nephrotoxicity and rhabdomyolysis. The effects of tubular necrosis have been attributed to the fosfolipasis A2 that determines the blocking of the Na^+ /glucose cotransport at the level of the apical region of the proximal tubule cell [1, 8].

CONCLUSIONS

1. The inoculation of bee venom as individual 0,3 mg doses on the rabbits under study made evident the progressive increase in the serum level of transaminases (ALT and AST) and of urea which demonstrated the insidious and slow occurrence of the hepatotoxicity and renal dysfunction, with even low venom doses.

2. The inoculation of the bee venom as 0.6 and 1.2 mg doses per rabbit revealed the quick increase after 2-6 h of the serum transaminases (AST and ALT) with a recovery after 24 h. but also an increase in ALP 24 h after inoculation which is indicative of the occurrence of some reversible hepatocellular lesions (it is to be elucidated the ALP increase to originate in either colangiocellulare or corticisuprarenal lesions.)

3. The quick increase in the urea serum level when doses of 0.6 and 1.2 mg venom are applied per rabbit followed by the recovery of the normal values after 24 h. is indicative of the acute renal dysfunction caused by the transient reduction of the blood flux followed by the starting of some compensatory mechanisms of homeostasis recovery and cellular detoxification.

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