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SERUM CONCENTRATIONS OF SELECTED ACUTE PHASE PROTEINS AND ENZYME ACTIVITIES AFTER INJECTION OF A COMBINED MINERAL PREPARATION IN CALVES

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The objective of this study was to evaluate the possible effects of various forms of injections of a combined mineral preparation in calves on changes in serum concentrations of selected acute phase proteins – haptoglobin (Hp) and serum amyloid A (SAA), as well as on changes in the activity of selected enzymes – creatine kinase (CK) and aspartate amino-transferase (AST). The changes in serum concentrations and activities of the aforementioned variables were evaluated after administration of the recommended therapeutic dose of the mentioned preparation and its repeated administration, and compared with changes recorded after a double therapeutic dose and its repeated administration. Analyses showed a more marked increase of mean SAA concentrations after intramuscular and subcutaneous injections of the higher preparation dose. A gradual insignificant increase in Hp serum concentrations was observed. The most significant changes were recorded in increasing serum activities of CK and AST after intramuscular injections of higher dose of the remedy ($P<0.001$ and $P<0.01$, respectively). Presented results indicate that tissue injury and muscle damage caused by repeated injections, predominantly at higher doses of drugs, may result not only in increased activities of some enzymes, but also in more marked changes in serum concentrations of certain acute phase proteins, particularly serum amyloid A.

Key words: calves, enzymes, haptoglobin, injections, serum amyloid A

INTRODUCTION

For many drugs, injection is the best and common method of administration to an animal. The most widely used methods of parenteral drug administration to animals are intramuscular and subcutaneous injections. However, if the proper technique is not used, or the injected products contain irritating vehicles, an injection has the potential to do harm due to considerable tissue damage, scar tissue, or abscesses that could cause the animal pain and suffering. Although there is a general tendency to produce less irritant drugs, some drugs

administered to cattle, predominantly to calves, may cause local reactions at the site of injection, which in some cases may result in a generalized inflammatory response of the body (Kováč and Nagy, 2007). Repeated injections and large injection volumes may promote a more marked tissue damage (Kern, 1987). Several methods have been used to measure tissue reactions after drug injections (clinical, histopathological, biochemical, ultrasound scanning) (Rasmussen and Svendsen, 1976; Banting, 1991). From the above mentioned methods, clinical methods were presented as less applicable and more subjective for examining tissue reactions after injections. It has also been shown that deep intramuscular injection can cause serious tissue damage without visible signs of pain (Luthman and Jacobsson, 1989). Diness (1985) and Lefebvre *et al*. (1994) reported that measuring the activity of muscle specific enzymes, especially serum creatine phosphokinase (CK) and aspartate amino-transferase (AST) provides the most specific method indicating muscle damage after injection of drugs. A further possibility to monitor muscle damage of various origins, including those after injection administration, could be measuring of changing concentrations of acute phase proteins (APPs) in the blood serum.

Serum concentrations of acute phase proteins are known to increase after microbial infections and following inflammatory stimulus, starvation or stress, as well as in neoplastic and traumatic disorders (Gabay and Kushner, 1999; Gruys *et al*., 2005). These serum proteins are likely to play an important role in the nonspecific defense mechanism of animals against microbial infection (Steel and Whitehead, 1994). On the other hand, APPs can also be detected in response to tissue injury induced by non-infectious, for example physical, or chemical events (Rath, 2005). However, little is known about the changes of the acute phase reactants after administration of drugs, which may act as a tissue irritant, and (particularly after intramuscular and subcutaneous injection) may cause local tissue injury. Therefore, we hypothesized that tissue injury and inflammation of such origin may also initiate an acute phase response. Apart from the events at the site of inflammation, such as increased vascular permeability and infiltration of tissue by phagocytic cells, humoral factors are also released, which produce a number of systemic mediators, including those that stimulate the hepatocytes into synthesis of acute phase reactants (Murata *et al*., 2004). In bovine medicine, haptoglobin (Hp) and serum amyloid A (SAA) are the major acute phase proteins, which concentrations are elevated in some important bovine diseases (Eckersall, 2006).

This work was aimed at the evaluation of possible effects of various forms of injections of the combined mineral remedy containing calcium on changes in serum concentrations of selected acute phase proteins – haptoglobin and serum amyloid A in calves, as well as on changes in the activities of selected enzymes – creatine kinase and aspartate amino-transferase. The changes in serum concentrations and activities of the aforementioned variables were evaluated after the administration of a commonly recommended therapeutic dose of the preparation by its repeated administration, and were compared with changes recorded by the double therapeutic dose and its repeated administration.

MATERIAL AND METHODS

Into the evaluation were included 18 clinically healthy calves of a low-land black spotted breed and its crossbreeds at the age of 4 – 6 months. The calves were housed in the Clinical Department for Ruminants of the University of Veterinary Medicine in Košice (Slovak Republic). They were fed hay and concentrates, and water freely *ad libitum*. Before any evaluation, the calves were allowed a one week settling period. A combined mineral preparation on the base of organic salts of calcium, magnesium and phosphorus was administered to calves. Animals were divided into 2 groups: calves in Group I. (n = 9) received repeatedly the basic therapeutic dose of the mentioned preparation (0.2 mL/kg); calves from Group II (n = 9) were repeatedly given a double therapeutic dose of the preparation (0.4 mL/kg). In both groups of animals, in three calves we performed the application of the remedy by *intra venam* (i.v.) injection to *v. jugularis*, three calves were *intramuscularly* (i.m.) injected into the neck muscle, and to three calves the preparation was administered *subcutaneously* (s.c.) behind the *scapula*. Repeated administration of the aforementioned drug was performed on the 7th day after the first administration. Injection sites were inspected and palpated after the administration of the preparation for the evaluation of possible local reactions after injections. Clinical reactions such as swelling or pain at the injection sites were evaluated.

The sample collections for analyses of monitored variables in conjunction with the administration of the remedy are designed in Table 1.

Table 1. Scheme of administration of the remedy and sample collections

Days	Sample collection	Description
1.	zero sampling	before the administration
	1st administration of the drug	
2.	1st sampling	24 hours after the 1st administration
3.	2nd sampling	48 hours after the 1st administration
8.	3rd sampling	7 days after the 1st administration
	2nd adminstration of the drug	
9.	4th sampling	24 hours after the 2nd administration
10.	5th sampling	48 hours after the 2nd administration
15.	6th sampling	7 days after the 2nd administration

Blood was collected by direct puncture of *v. jugularis*. Blood serum was analysed for selected APPs – haptoglobin (Hp) and serum amyloid A (SAA), and selected enzymes – creatine kinase (CK) and aspartate aminotransferase (AST). Hp was assessed using a commercial colorimetric kit (Tridelta Development, Ireland) based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH. SAA was analysed by the method of

sandwich enzyme linked immunosorbent assay using commercial ELISA kit (Tridelta Development, Ireland) on microplates. Readings of absorbance and the consecutive calculation of final concentrations of both evaluated APPs were performed on an automatic microplate reader Opsys MR (Dynex Technologies, USA). The enzyme activities of CK and AST were determined by the spectrophotometric method using commercial diagnostic kits (Randox) on the automatic biochemical analyser ALIZE (Lisabio, France).

Statistical evaluation of the results was performed by assessment of means (x) and standard deviations (sd) in each group of calves and for each form of injection. The significance (P) of differences in the means of corresponding variables was evaluated by one way analysis of variance (ANOVA). The significance of differences between the sample collections using Tukey-Kramer Multiple Comparisons Test was evaluated at the same time. Statistical analyses was done with the GraphPad Instat V2.04 software.

RESULTS

The obtained results given as means, standard deviations, and evaluation of the significances of differences in means are presented in Tables 2 – 5.

For the serum concentrations of Hp (Table 2) after the intravenous injection of the basic, as well as the double dose of the preparation we did not record significant changes during studied period. Significant changes in Hp concentrations during the monitored period were found after the i.m. administration of the basic dose of the preparation ($P<0.01$). Mild increase of Hp concentrations compared with values found before and 24 hours after the repeated injection was recorded 48 hours and 7 days after the repeated administration of the basic terapeutic dose. An insignificant increase in Hp serum concentrations after intramuscular injection of a double therapeutic dose was observed from the 48th hour after administration. Tendency of further elevation of mean Hp concentrations was recorded also after repeated administration (4th and 5th samplings). Insignificant changes in serum Hp concentrations were found also after subcutaneous injections of both the basic and higher dose of the remedy. These changes were characterised by an increase of mean Hp concentrations 24 and 48 hours after administration.

Throughout the period under study, the dynamics of serum SAA concentrations was not significantly influenced by intravenous administration neither of basic dose, nor of double therapeutic dose of the preparation (Table 3). After i.m. injection of the recommended therapeutic dose of the preparation, an insignificant dynamics of changes was found in serum SAA concentrations. A mild increase of SAA concentrations was observed 24 hours after the first injection, and 24 and 48 hours after repeated administration of the remedy. By administration of the higher therapeutic dose similar changes were recorded in serum concentrations of SAA with a marked increase of its mean concentrations 24 hours after the first administration and a further moderate increase 48 hours after the injection. While subcutaneous injections of the basic dose of the monitored preparation did not have a significant influence on the serum

Table 2. Changes in the concentrations of Hp in blood serum of calves after administration of combined mineral preparation (mg/mL)

Group			Sample collection						Anova P
			0.	1.	2.	3.	4.	5.	6.	
i. v.	I.	x	0.34	0.31	0.16	0.08	0.06	0.08	0.07	n. s.
		± sd	0.26	0.16	0.14	0.10	0.04	0.03	0.01	
	II.	x	0.29	0.16	0.04	0.29	0.46	0.33	0.07	n. s.
		± sd	0.27	0.13	0.03	0.40	0.42	0.26	0.01	
i. m.	I.	x	0.36^{a,b}	0.30	0.16	0.06^{a}	0.03^{b}	0.11	0.10	<0.01
		± sd	0.18	0.12	0.12	0.01	0.01	0.03	0.04	
	II.	x	0.07	0.09	0.48	0.54	0.63	0.73	0.14	n. s.
		± sd	0.01	0.02	0.31	0.55	0.39	0.70	0.11	
s. c.	I.	x	0.53	0.74	0.34	0.25	0.44	0.53	0.44	n. s.
		± sd	0.32	0.71	0.19	0.16	0.66	0.62	0.45	
	II.	x	0.08	0.06	0.15	0.03	0.13	0.14	0.09	n. s.
		± sd	0.01	0.02	0.13	0.01	0.02	0.07	0.03	

The same superscripts in rows mean statistical differences in means between the sample collections: a, b – P< 0.05
I – group of calves injected with basic therapeutic dose of the preparation
II – group of calves injected with double therapeutic dose of the preparation

Table 3. Changes in the concentrations of SAA in blood serum of calves after administration of combined mineral preparation (μg/mL)

Group			Sample collection						Anova P
			0.	1.	2.	3.	4.	5.	6.	
i. v.	I.	x	36.83	38.57	48.47	14.37	15.53	15.97	12.92	n. s.
		± sd	18.20	18.60	31.20	5.20	2.40	8.10	4.90	
	II.	x	20.86	16.59	18.12	33.90	27.83	25.63	12.22	n. s.
		± sd	30.10	16.60	13.50	20.20	19.60	11.70	9.60	
i. m.	I.	x	22.70	36.44	30.75	8.60	14.70	22.80	10.68	n. s.
		± sd	23.80	44.10	22.40	3.20	3.00	9.70	5.20	
	II.	x	1.33	41.87	53.97	44.13	37.67	42.30	9.91	n. s.
		± sd	1.80	30.80	42.00	17.40	31.50	34.90	4.90	
s. c.	I.	x	54.47	55.18	54.07	23.60	33.37	25.80	24.65	n. s.
		± sd	38.90	42.50	30.50	11.90	32.00	19.30	24.80	
	II.	x	1.40	13.26	33.67	11.86	9.73	19.05	9.24	n. s.
		± sd	1.90	8.30	28.70	3.80	3.20	13.00	12.50	

I – group of calves injected with basic therapeutic dose of the preparation
II – group of calves injected with double therapeutic dose of the preparation

Table 4. Changes in the activities of CK in blood serum of calves after administration of combined mineral preparation (μkat/L)

Group			Sample collection						Anova P
			0.	1.	2.	3.	4.	5.	6.	
i. v.	I.	x	4.53	6.62	5.30	5.92	5.89	6.47	8.36	n. s.
		± sd	1.30	2.30	1.10	0.60	0.80	0.60	2.60	
	II.	x	6.89	8.44	7.03	9.65	8.79	8.47	7.03	n. s.
		± sd	0.60	2.20	0.90	3.50	2.50	2.20	0.80	
i. m.	I.	x	12.12	17.53	10.16	19.20	19.70	10.57	9.35	<0.05
		± sd	6.90	6.50	2.40	1.80	1.80	0.50	1.60	
	II.	x	5.86[1]	38.0[1,2,3,4,5,6]	11.24[2]	6.21[3]	10.96[4]	6.14[5]	4.79[6]	<0.001
		± sd	0.90	9.80	1.80	2.60	2.80	1.10	1.00	
s. c.	I.	x	8.92	49.57	43.70	21.70	22.07	16.90	8.06	n. s.
		± sd	4.20	45.50	32.20	5.60	6.10	2.40	1.30	
	II.	x	6.52	31.53	25.30	8.38	29.87	19.83	6.69	n. s.
		± sd	1.00	8.60	12.80	3.20	27.10	10.90	1.20	

The same superscripts in rows mean statistical differences in means between the sample collections: 1, 2, 3, 4, 5, 6 – P<0.001
I – group of calves injected with basic therapeutic dose of the preparation
II – group of calves injected with double therapeutic dose of the preparation

Table 5. Changes in the activities of AST in blood serum of calves after administration of combined mineral preparation (μkat/L)

Group			Sample collection						Anova P
			0.	1.	2.	3.	4.	5.	6.	
i. v.	I.	x	1.28	1.36	1.39	1.36	1.57	1.52	1.56	n. s.
		± sd	0.30	0.40	0.30	0.04	0.01	0.10	0.08	
	II.	x	$1.38^{a,A}$	1.49^{b}	1.45^{c}	1.64	1.87^{a}	$1.98^{A,b,c}$	1.67	0.01
		± sd	0.09	0.07	0.07	0.20	0.20	0.20	0.20	
i.m.	I.	x	3.32	3.35	3.47	2.48	2.80	2.25	2.44	n. s.
		± sd	3.20	2.40	1.70	0.50	0.50	0.40	1.40	
	II.	x	$1.30^{A,a}$	$2.41^{A,b,c}$	2.20^{a}	1.35^{b}	1.68	1.61	1.36^{c}	0.01
		± sd	0.10	0.30	0.30	0.40	0.30	0.40	0.30	
s. c.	I.	x	1.65	3.61	4.59	2.20	2.48	2.26	1.73	n. s.
		± sd	0.50	2.60	3.40	0.60	0.60	0.70	0.30	
	II.	x	1.51	1.83	2.12	1.91	2.36	2.44	1.93	n. s.
		± sd	0.40	0.40	0.20	0.80	0.50	0.60	0.60	

The same superscripts in rows mean statistical differences in means between the sample collections: a, b, c – P 0.05; A – P<0.01
I – group of calves injected with basic therapeutic dose of the preparation
II – group of calves injected with double therapeutic dose of the preparation

concentrations of SAA, after administration of the higher dose an insignificant tendency of increasing mean SAA concentrations after the first, as well as after the second injection was observed.

The activity of CK in blood serum was not significantly influenced by i.v. administration of basic, nor double therapeutic doses of the preparation (Table 4). Significant changes of CK activity were recorded after the intramuscular injection of the basic ($P<0.05$) and higher dose of the preparation ($P<0.001$). While after the administration of the recommended basic dose a moderate increase of CK activity, after the administration of a double dose a marked increase of its activity ($P<0.01$) was found 24 hours after the first injection (1st sampling). A similar, but not significant trend of increasing CK activity was found also after s.c injection of the basic, as well as higher dose of the monitored remedy. A marked increase of CK activity in both groups of calves was recorded 24 hours after the first administration.

Intravenous administration of the recommended basic therapeutic dose of the monitored preparation had no significant influence on the activity of blood serum AST (Table 5). After administration of the higher dose of the preparation, we observed significant changes in the activity of this enzyme ($P<0.01$), particularly after repeated injections. After intramuscular injections of the basic dose of the remedy an insignificant dynamics was recorded in AST activities during the whole period under study. More significant changes with increasing activity were found after i.m. injection of the double dose of the preparation ($P<0.01$). A significant increase of AST activity ($P<0.01$) was recorded 24 hours after the first administration. In the case of subcutaneous administration we observed a tendency of increasing AST activities both by basic and double dose of the preparation. However, the changes of activity of this enzyme were not significant.

After intravenous administeration, we did not find any clinical reactions and we did not observe local signs of intolerance of the preparation both by basic and double therapeutic doses even after repeated administration. Intramuscular injection of basic, as well as double dose of the preparation induced a temporal mild swelling at the injection site in 2 animals. These swellings disappeared in the course of few days. Marked signs of pain at the injection sites were not recorded. More marked differences in relation to the dose and frequency of the applications were not observed. After subcutaneous injection of the monitored remedy, mild temporal swelling of the injection site was found by the use of basic, as well as double therapeutic dose in 2 animals. These signs disappeared during the following days. Differences in clinical reactions at the injection sites in relation to the dose and repeating of the administrations were not observed.

DISCUSSION

The localised inflammation and tissue damage is believed to lead to the release of messengers from macrophages in the damaged area into the bloodstream, which then act upon the liver and stimulate the synthesis of acute phase proteins (Laurell, 1985). However, there have been only a few reports about the increased production of acute phase proteins in cases of local tissue injury or

muscle damage caused by parenteral administration of drugs, particularly by their repeated administration. Conner *et al. (*1988) reported that serum from calves receiving subcutaneous injections of oil of turpentine (a tissue irritant) has been shown to have elevated concentrations of α_1-antitrypsin, ceruloplasmin, fibrinogen, haptoglobin and seromucoid. Similar findings were demonstrated for Hp and C-reactive protein (CRP) in pigs (Lampreave *et al.,* 1994; Eckersall *et al.,* 1996). The presented results indicate that parenteral administration of drugs, commonly used in veterinary practice particularly in higher doses, may cause also an increase in serum concentrations of certain acute phase proteins. Predominantly intramuscular and subcutaneous injections may cause systemic inflammatory responses characterised by increased serum acute phase concentrations because of tissue injury and muscle damage caused by the injections. However, we observed major differences between the evaluated acute phase proteins, as well as between the commonly used ways of application in the responses of animals to injections. After intramuscular and subcutaneous injections we recorded more significant changes than after intravenous administrations. The obtained changes in SAA and Hp concentrations indicate that i.m. injections may cause more marked tissue reactions. While 24 hours after i.m. injection of the higher dose of the evaluated preparation we found a more than 30 fold increase in mean SAA concentrations and a 9.5 fold increase after s.c. injections. In the case of intravenous administration of the same dose of the preparation, the SAA values remained unchanged. By evaluation of serum Hp concentrations 48 hours after i.m. injection of double dose of the monitored preparation we found about 7 times higher values than prior to the administration. On the other hand, after s. c. injection we recorded only a slight increase of Hp concentrations (approximately a 2 fold increase). The more intensive response of the body to intramuscular injection of the higher dose of the drug, characterised by an increase of Hp and SAA concentrations, may be caused by a more serious local tissue reaction at the site of i.m. injection, than after subcutaneous administration. Luthman and Jacobsson (1989) reported also that intramuscular administration of drugs, particularly deep i.m. injections can cause more serious tissue damage.

During the time under study, we observed major differences also in the dynamics of changes of serum acute phase protein concentrations. While the increase of mean Hp concentrations after i.m. injection of the higher dose of the evaluated remedy was gradual with a further gradual increase after repeated administration, for serum concentrations of SAA we recorded a rapid, more than 30 fold increase 24 hours after the first administration. These differences may be the consequence of a different initiation of the production of various acute phase proteins and their various reactivity to inflammatory stimuli. Hp is characterised by a later increase in serum concentrations after stimulus, which remains elevated for a longer period. On the other hand, SAA is a rapidly reacting acute phase protein, which is characterised by a dramatic increase in serum concentrations after the inflammatory stimulus and a relatively rapid normalisation (Petersen *et al.,* 2004). Gruys *et al.* (1993) reported also that SAA reacts faster than haptoglobin in response to an acute phase protein inducing event. Moreover, there are

numerous differences in the inflammatory responses to various external or internal stimuli between the animals, some of them respond markedly, other have moderate or minor responses. Higher values of standard deviations obtained in both groups of calves reflect the different reactivity of acute phase proteins characterised by a different rate of increase of mean Hp and SAA concentrations after i.m. and s.c. injections. The wider range of individual values suggests also the differences in the variability of animals reacting to injury.

Comparisons between the administration of the basic and double dose of the preparation indicate that the changes in serum acute phase proteins are influenced also by the injected volume of the drug. After i.m. injection of the higher dose we recorded significant changes in the concentrations of SAA, characterised by increasing values during the evaluation. Presumably, greater doses of administered drugs cause more extensive tissue damage and therefore higher concentrations of SAA in the blood. Pepys and Baltz (1983) reported in humans, that the increase in serum concentrations of C-reactive protein, as the most useful indicator of inflammation, reflects the extent of active tissue damage. According to Conner *et al.* (1988), concentrations of haptoglobin vary with the dose of applied tissue irritant, as after administration of the higher dose of turpentine a marked increase in the concentrations was recorded. Although our results showed a more marked increase of mean serum Hp concentrations after repeated i.m. injections of the higher dose of the remedy, this increase was gradual and insignificant.

The presented results indicate that repeated injections may lead to important changes in enzyme activities of creatine kinase and aspartate aminotransferase, caused by *post injection* tissue injury. Gueorgieva *et al.* (1998) and Kanelov (2008) reported that i.m. injections may result in increased enzyme activity of serum CK. Similar to the results obtained in serum concentrations of Hp and SAA, enzyme activities of CK and AST showed the most significant changes after i.m. and s.c. injections of the higher dose of the evaluated preparation. Intravenous administration of the preparation caused only a mild insignificant increase of enzyme activity. Pyörälä *et al.* (1994) reported that the medicinal products causing severe tissue irritation at the injection site increased serum CK activity in cattle by up to 10 – 16 times. During our evaluation, 24 hours after i.m. injection of the double dose of the preparation we found approximately a 6.5 fold increase of CK activity, and an about a 5 fold increase of its activity after s.c. injection, with consistent decrease of enzyme activity in both cases. The reduction of the serum CK activity after peak values could be explained by the direct elimination and by intravascular inactivation (Lefebvre *et al.,* 1996). A similar significant increase of enzyme activity after i.m. and s.c. injections, as well as after i.v. administration of a higher dose of the remedy was observed also for AST. However, this increase was less marked (approximately a 1.5 increase) compared with changes obtained in creatine kinase activities. Pavlata *et al.* (2001) reported also that in cattle with muscular damage, there is an increase in CK activity, as well as in AST and lactate dehydrogenase activities. According to Duncan and Prasse (1997) and Meyer and Harvey (1998), AST is a less specific indicator of muscle damage, because is released also from other damaged cells, particularly from

hepatocytes. However, it is relevant to the diagnosis and differential diagnosis of muscular damage.

In conclusion, presented results indicate that tissue injury and muscle damage caused by repeated injections, predominantly of higher doses of drugs, may result not only in increased activities of some enzymes, but also in more marked changes in serum concentrations of certain acute phase proteins, particularly serum amyloid A. Therefore, by simultaneous injections of drugs and collections of blood samples in laboratory diagnosis, as well as by evaluation of the efficacy of treatment, it is important to keep in mind that the activities of some enzymes and the concentrations of some acute phase proteins may be influenced also by performing common veterinary interventions.

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KONCENTRACIJA ODABRANIH PROTEINA AKUTNE FAZE I AKTIVNOST POJEDINIH ENZIMA U SERUMU TELADI POSLE INJEKCIONIH APLIKACIJA MINERALNIH PREPARATA

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SADRŽAJ

Cilj ovih ispitivanja je bio da se utvrde mogući efekti injekcione aplikacije različitih oblika kombinovanih mineralnih preparata na koncentraciju odabranih

proteina akutne faze u serumu teladi: haptogobina (Hp) i serumskog amiloida (SAA), kao i na aktivnost kreatin kinaze (CK) I aspartat amino-transferaze (AST). Pomenuti efekti su utvrđivani posle jednokratne i ponovljene aplikacije terapijskih doza pojedinih preparata i upoređivani su sa efektima registrovanim nakon jednokratne i ponovljene aplikacije dvostruko većih doza.

Postignuti rezultati su ukazali na značajno povećanje koncentracije SAA posle subkutane i intramuskularne aplikacije preparata. Koncentracija haptoglobina u serumu je takođe bila povećana ali ovo povećanje nije bilo statistički značajno. Najznačajnije povećanje je registrovano određivanjem aktivnosti SK i AST posle intramuskularne aplikacije većih doza preparata ($p<0,001$ i $p<0,01$, respektivno). Naši rezultati, takođe ukazuju, da povrede tkiva i oštećenja mišića usled ponovljenih intramuskularnih aplikacija većih doza rezultira, ne samo povećanjem aktivnosti pojedinih enzima već i povećanjem koncentracije serumskog amiloida kao proteina akutne faze.