Investigations on Blood Activities of Some Enzymes in Dogs after Acute Intoxication with the Carbamate Insecticide Carbofuran

Rumen Binev*, Ivan Valchev, Anton Russenov, Yordan Nikolov

Faculty of Veterinary Medicine; Trakia University, Student's Campus, 6000 Stara Zagora, Bulgaria

Abstract

Experiments for monitoring of changes in blood enzyme activities were carried out in dogs after acute intoxication with the carbamate insecticide carbofuran (Carbosan 35 CT).

The studies involved one control and 6 experimental groups of dogs (total n=42), treated once orally with increasing doses of the preparation via oesophageal probe: 0.525 mg/kg (experimental group I), 1.05 mg/kg (experimental group II), 2.1 mg/kg (experimental group III), 3.5 mg/kg (experimental group IV), 5.25 mg/kg (experimental group V) and 10.5 mg/kg (LD_{50}), (experimental group VI), corresponding to 1/20, 1/10, 1/5, 1/3, 1/2 and LD_{50}, oral doses for albino rats.

Blood samples were obtained from v. antebrachi cephalica or v. jugularis in the course of 3 consecutive days prior to the treatment (hours -48, -24 and 0) and on post treatment hours 1, 3, 5, 7, 24 and 48 from all groups for analysis of acetylcholinesterase (AChE), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), amylase (AMY), gamma-glutamyltransferase (γ-GT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

It was established that the tested carbamate insecticide provoked lower blood activity of AChE and increased the levels of ASAT, ALAT, AMY, γ-GT, AP and LDH between post treatment hours 1 and 7; afterwards, the studied parameters regained the respective control values.

Keywords: intoxication, carbamate insecticide, carbofuran, blood enzyme activity, dogs.

1. Introduction

From all pesticides used for agricultural protection, the group of insecticides is the largest one, and within it, organocarbamate insecticides (OCI) are the most utilised [1-3]. This is due to their high efficacy and broad spectrum of application as insecticide, nematocide and acaricide substances [4-6]. At the same time, they are outlined with a high toxicity, hence the high risk for acute intoxications in animals [2, 7-9].

More than half of intoxication cases in domestic animals and game species reported in Bulgaria and abroad are due to carbamate pesticides [6, 10-14].

Carbofuran is the most commonly used OCI. It belongs to the group of highly toxic compounds (LD_{50}<50 mg/kg), has no specific odour and preserves its toxicity for a long time in the environment [15]. It is widely used by reason of its high bioactivity against harmful insects, which entails its presence in waters, air, soils and explains the numerous intoxications in domestic [11, 13, 16-18] and wild [9, 12, 19-22] animals. By now, the greatest existing toxicological risk comes from the use of carbofuran for restriction of rodent populations [8, 21, 23], under the form of baits – an illegal practice [6], particularly in cases of deliberate intoxications of stray dogs and cats [9, 25, 26].

The existing literature data demonstrate that acute intoxication with OCI results in substantial inhibition of the activity of acetylcholinesterase, which could attain up to 80% [26], even 90% [27]

* Corresponding author: Rumen Binev, Tel. 359-42-699-530, Email: binew@abv.bg

of usual blood levels. In general, clinical signs of intoxication with preparations from this group appear after inhibition of 50-60% of AChE [1]. Carbamates bind to the enzyme via carbamylation, which blocks the ester bond [15, 28]. A specific feature of OCI intoxication is that AchE inhibition is a reversible process unlike the action of organophosphates [22]. The spontaneous reactivation of cholinesterase activity depends both on the animal species and the amount of ingested toxic substance [5, 16]. This determines the contradictory literature data about the beginning of the enzyme inhibition, its duration and the time for recovery of AChE level after the challenge.

Data reported on the influence of carbamate pesticides on blood enzyme AChE activity are mainly in spontaneous intoxications [4, 6, 10, 17, 18, 22, 29].

In previous studies of ours on changes in activities of some blood enzymes in quails with experimental acute intoxication with the carbamate insecticide carbofuran [30], we established lower activity of acetylcholinesterase, and increased levels of ASAT, ALAT, ALP, creatine kinase and slight variations in γ-GT.

The purpose of the present study was to determine the toxic effect of the carbamate insecticide carbofuran in dogs through monitoring of changes of some blood enzymes after experimental acute intoxication with the preparations.

2. Materials and methods

Experimental animals. The experiments were carried out on 42 dogs, at the same age and weight. Thirty days prior to the study they were kept in individual cages under the same conditions in compliance to hygienic microclimatic and feeding norms. The animals were fed canine food for adults and had free access to water.

*Tested substance.*The experimental intoxication was done with the commercial preparation Carbosan 35 CT (Agro Science, USA), containing 350 mg carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate) in 1 mL, with oral LD_{50} for albino rats = 10.5 mg/kg. The preparation was applied once orally via an oesophageal probe, two hours before feeding (at 6.00 AM).

Experimental design. The dogs were divided into 7 groups with equal number from both genders: one control and six experimental (6 dogs in each), treated on hour 0 with increasing single doses as followed: experimental group I: 0.525 mg/kg (1/20 $LD_{50,}$ for albino rats); experimental group II (1/10 $LD_{50,}$ for albino rats): 1.05 mg/kg; experimental group III: 2.1 mg/kg (1/5 LD_{50}); experimental group IV: 3.5 mg/kg (1/3 LD_{50}); experimental group V: 5.25 mg/kg (1/2 LD_{50}), and experimental group VI: 10.5 mg/kg (LD_{50}).

Blood samples were obtained from v. antebrachi cephalica or v. jugularis in the course of 3 consecutive days prior to the treatment (hours -48, -24 and 0) and on post treatment hours 1, 3, 5, 7, 24 and 48 from all groups for analysis of acetylcholinesterase (AChE), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), amylase (AMY), gamma-glutamyltransferase (γ-GT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) using commercial kits Cyba Corning (Bayer Diagnostics Ltd, Germany) and automated biochemical analyser Olympus AU 600 (Japan).

All results were processed with statistical software (Statistica 6.0 for Windows, Stat Soft Inc. USA, 1993). The significance of differences between treated groups and untreated controls were evaluated by ANOVA. The level of statistical significance was $P<0.05$.

Results and discussion

The analysis of serum activity of cholinesterase (Fig. 1) revealed decrease of the studied blood analyte in all treated groups by the 1st hour as followed: 1943±44 U/l ($p<0.05$) in group I; 783±48 U/l ($p<0.001$) in group II; 538±28 U/l ($p<0.001$) in group III; 482±22 U/l ($p<0.001$) in group IV and 233±18 U/l ($p<0.001$) in group V as compared to controls – 2761±174 U/l. These values were the lowest detected activities for the enzyme. The recovery of enzyme activity in group I occurred by post treatment hour 3, in group II – by hour 5 and in groups III, IV and V – by post treatment hour 7.

The activity of aspartate aminotransferase (ASAT) in blood (Fig. 2) increased one hour after the treatment in all experimental groups. The studied parameter attained highest values by the 3rd hour: 57.3±3.8 U/l ($p<0.001$) in group I, 75.8±5.2 U/l

(p<0.001) in group II, 94.3±8.5 U/l (p<0.001) in group III, 110.4±9.8 U/l (p<0.001) in group IV and 122.2±11.4 U/l (p<0.001) in group V vs control levels (13.8±0.9 U/l). The enzyme levels in in groups I, II and III returned to normal by the 24th hour, and in the other two groups – by the 48th hour.

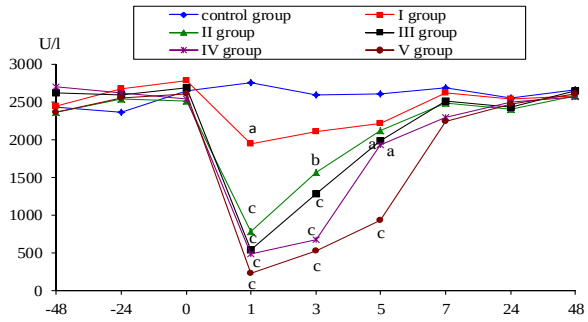

Figure 1. Changes in blood acetylcholinesterase activity in dogs – untreated (control) and treated with carbofuran at doses 0.525 mg/kg (1/20 LD_{50}), (group I), 1.05 mg/kg (1/10 LD_{50}), (group II), 2.1 mg/kg (1/5 LD_{50}), (group III), 3.5 mg/kg (1/3 LD_{50}) (group IV) and 5.025 mg/kg (1/2 LD_{50}), (group V): $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.

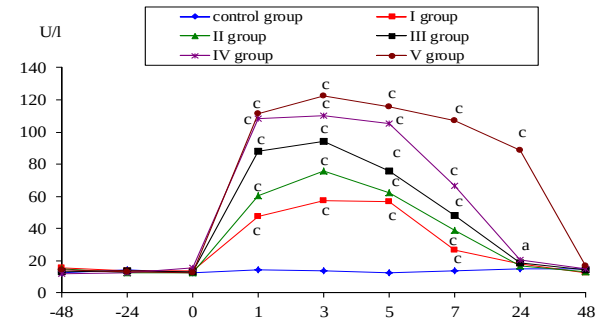

Figure 2. Changes in blood aspartate aminotransferase (ASAT) activity in dogs – untreated (control) and treated with carbofuran at doses 0.525 mg/kg (1/20 LD_{50}), (group I), 1.05 mg/kg (1/10 LD_{50}), (group II), 2.1 mg/kg (1/5 LD_{50}), (group III), 3.5 mg/kg (1/3 LD_{50}) (group IV) and 5.025 mg/kg (1/2 LD_{50}), (group V): $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.

The enzyme activity of alanine aminotransferase (ALAT) (Fig. 3) followed a similar trend of change in all challenged animals by the 1st hour. In groups I and II, changes were observed only on this period with activities of 25.8±2.1 U/l (p<0.05) and 30.3±2.8 U/l (p<0.05), respectively vs control level (18.8±1.7 U/l). In the other groups (third, fourth and fifth), ALAT levels remained elevated for a longer period with peak values by the 3rd hour – 44.6±3.9 U/l (p<0.01), 66.4±5.2 U/l (p<0.001) и 82.3±6.4 U/l (p<0.001), respectively as compared to controls – 21.3±2.2 U/l. In group III ALAT activity returned to normal by post treatment hour 24, in group IV: by the 48th hour and in group V persisted elevated after the end of the study period.

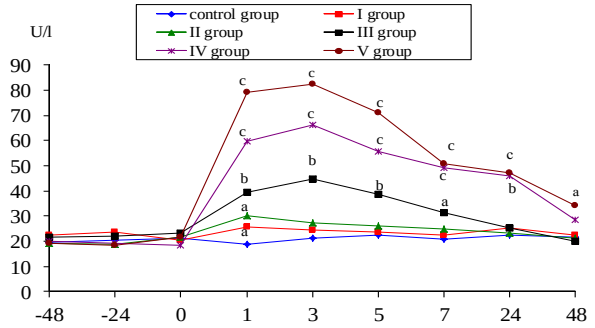

Figure 3. Changes in blood alanine aminotransferase (ALAT) activity in dogs – untreated (control) and treated with carbofuran at doses 0.525 mg/kg (1/20 LD_{50}), (group I), 1.05 mg/kg (1/10 LD_{50}), (group II), 2.1 mg/kg (1/5 LD_{50}), (group III), 3.5 mg/kg (1/3 LD_{50}) (group IV) and 5.025 mg/kg (1/2 LD_{50}), (group V): $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.

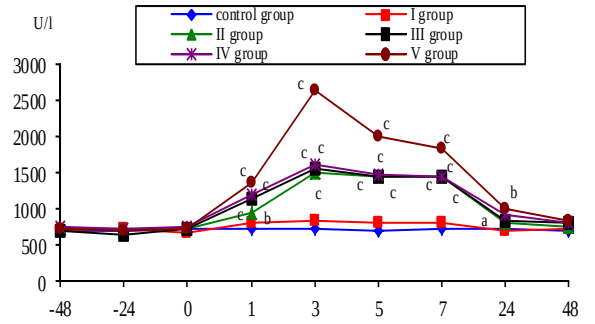

Figure 4. Changes in blood amylase (AMY) activity in dogs – untreated (control) and treated with carbofuran at doses 0.525 mg/kg (1/20 LD_{50}), (group I), 1.05 mg/kg (1/10 LD_{50}), (group II), 2.1 mg/kg (1/5 LD_{50}), (group III), 3.5 mg/kg (1/3 LD_{50}) (group IV) and 5.025 mg/kg (1/2 LD_{50}), (group V): $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.

The analysis of enzyme activities of amylase (Fig. 4) shoed increased concentrations by the first hour in groups II, III, IV and V as followed – 956±48 U/l (p<0.01), 1152±53 U/l (p<0.001), 1213±58 U/l (p<0.001) and 1368±54 U/l (p<0.001), respectively. Control activity by hour 1 was 718±39 U/l. Peak levels were detected by hour 3 – 1518±58 U/l (p<0.001), 1563±56 U/l (p<0.001), 1616±62 U/l (p<0.001) and 2638±93 U/l

(p<0.001) in groups II, III, IV and V respectively, while control dogs had an average AMY activity of 736±34 U/l. The blood level of amylase in groups II and III was normalised by hour 24, and of groups IV and V – by hour 48.

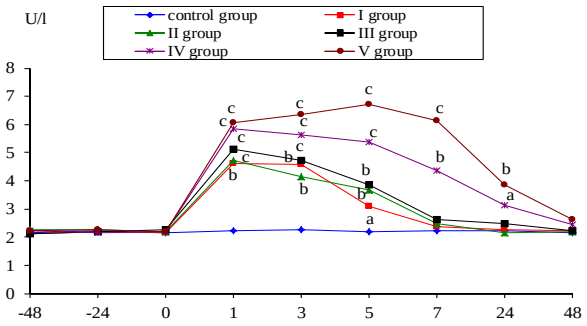

Figure 5. Changes in blood gamma-glutamyltransferase (γ-GT) activity in dogs – untreated (control) and treated with carbofuran at doses 0.525 mg/kg (1/20 LD_{50}), (group I), 1.05 mg/kg (1/10 LD_{50}), (group II), 2.1 mg/kg (1/5 LD_{50}), (group III), 3.5 mg/kg (1/3 LD_{50}) (group IV) and 5.025 mg/kg (1/2 LD_{50}), (group V): $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.

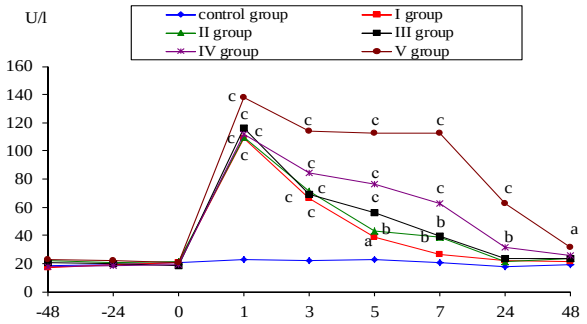

Figure 6. Changes in blood alkaline phosphatase (ALP) activity in dogs – untreated (control) and treated with carbofuran at doses 0.525 mg/kg (1/20 LD_{50}), (group I), 1.05 mg/kg (1/10 LD_{50}), (group II), 2.1 mg/kg (1/5 LD_{50}), (group III), 3.5 mg/kg (1/3 LD_{50}) (group IV) and 5.025 mg/kg (1/2 LD_{50}), (group V): $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.

In all treated groups, gamma glutamyltransferase (γ-GT) increased an hour after administration of the carbamate insecticide carbofuran (Fig. 5): 4.62±0.38 U/l in group I (p<0.01), 4.73±0.36 U/l in group II (p<0.001), 5.12±0.46 U/l in group III (p<0.001), 5.86±0.48 U/l in group I (p<0.001) and 6.08±0.53 U/l in group V (p<0.001) as compared to control levels – 2.22±0.18 U/l. These levels were the highest detected for groups I, II, III and IV, while peak γ-GT in group V occurred by hour 5 – 6.73±0.49 U/l (p<0.001), when control activity was 2.19±0.17 U/l. Blood γ-GT activities in the first three experimental groups were recovered by hour 7, while in groups IV and V – by the 48th h.

The changes in alkaline phosphatase activity (Fig. 6) was characterised with elevation in all groups treated with carbofuran by the 1st h, namely 105.3±8.4 U/l (p<0.001) in group I, 109.8±8.1 U/l (p<0.001) in group II, 116.3±8.8 U/l (p<0.001) in group III, 111.9±8.2 U/l (p<0.001) in group IV and 137.8±9.7 U/l (p<0.001) in group V vs untreated dogs – 22.6±2.4 U/l. These ALP activities were the highest for all groups. Activities of the enzyme in blood were restored by hour 7 (group I), hour 24 (groups II and III), hour 48 (group IV) while in group V remained statistically significantly higher throughout the entire period of the study.

Blood lactate dehydrogenase (Fig. 7) increased one hour after the challenge in all treated groups – 80.6±6.61 U/l (p<0.05), 98.3±6.80 U/l (p<0.01), 150.8±9.38 U/l (p<0.001), 156.4±9.38 U/l (p<0.001) and 198.2±8.79 U/l (p<0.001) in groups I, II, III, IV and V, respectively at the background of low values in controls (49.7±5.29 U/l). Except for group III, where peak activity occurred by hour 3 – 152.4±9.27 U/l (p<0.001 vs 48.5±4.92 U/l in controls) the activities measured on hour 1 were the highest for all treated dogs. LDH was restored by hour 5 in group I, by hour 24 in groups II, III and IV and by the 48 hour in group V.

Dogs treated at 10.5 mg/kg (LD_{50}), (experimental group VI) showed signs of intoxication after the 5th minute and died within 30 to 45 min after the treatment.

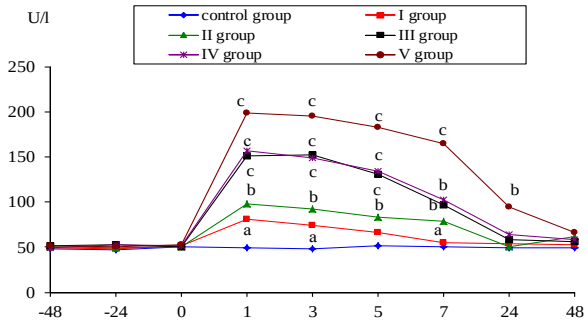

Figure 7. Changes in blood lactate dehydrogenase (LDH) activity in dogs – untreated (control) and treated with carbofuran at doses 0.525 mg/kg (1/20 LD_{50}), (group I), 1.05 mg/kg (1/10 LD_{50}), (group II), 2.1 mg/kg (1/5 LD_{50}), (group III), 3.5 mg/kg (1/3 LD_{50}) (group IV) and 5.025 mg/kg (1/2 LD_{50}), (group V): $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.

The serum acetylcholinesterase decreased on all experimental groups by the 1st hour regardless of the dose of tested preparation. At this time interval, the activities of the enzyme were the lowest. In dogs treated at 5.025 mg/kg (1/2 от LD_{50}), the reduction was more than 10-fold vs control measurements. AChE activity was restored by the 3rd hour in dogs treated at a dose equal to 1/20 LD_{50}, and by hour 5 in those treated at 1/10 LD_{50} – на 5-ия h. In all other groups (1/5 LD_{50}; 1/3 LD_{50} and 1/2 LD_{50} the recovery was observed 7 hours after the challenge with carbofuran.

These results for blood AChE activities showed a clear trend to reduction of activity, whose magnitude was dependent on the treatment dose. Most obvious changes were noticed one hour after the treatment. These data confirmed that the toxic effect of carbamate insecticides was the strongest in the first hours after ingestion of the poison, as observed in dogs [3, 18, 25, 27, 29], wild animals [8, 9, 17]. The beginning of acetylcholinesterase recovery by the 3rd hour in all groups with complete normalisation by hour 7 emphasises the well-known fact that the inhibition of the enzyme under the influence of carbamates (including carbofuran) is a reversible process, directly associated to the dose of applied pesticides and the species of experimental animals [23, 25, 27, 28].

The comparison of data from our studies [30] on cholinesterase activity in the blood of quails treated with similar doses of the preparation has shown that enzyme activity began to decrease in birds treated at 2.1 mg/kg (1/5 LD_{50}). In dogs, enzyme inhibition occurred after treatment at considerably lower doses – 0.525 mg/kg (1/20 LD_{50}) and especially at 1.05 mg/kg (1/10 LD_{50}). These species-specific features allowed affirming that quails were a more resistant species than dogs to carbamate intoxication. In addition, after the maximum tested dose 10.5 mg/kg (LD_{50}) carbofuran resulted in fatal outcome in all dogs, whereas quails treated at the same dose survived the intoxication.

The acute experimental intoxication with the carbamate insecticide carbofuran in dogs treated with increasing doses (1/20 LD_{50}, 1/10 LD_{50}, 1/5 LD_{50}, 1/3 LD_{50} and 1/2 LD_{50}) was accompanied with substantial changes in activities of transferases – ASAT, ALAT, γ-GT), ALP and LDH.

Our experiments have shown that the concentrations of both aminotransferase (ASAT and ALAT) increased in all experimental dogs proportionally to the treatment dose of carbofuran. The changes were detected by the 1st hour, with peak levels by hour 3. Blood activities increased about 4 times (ALAT) and about 8-9 times (ASAT) as compared to control measurements. The recovery of ASAT activity in dogs treated at 1/20 LD_{50}, 1/10 LD_{50}, and 1/5 LD_{50} occurred by the 24th hour after carbofuran administration and by the 48th hour in experimental groups that received 1/3 LD_{50} and 1/2 LD_{50} of the tested preparation. The same tendency in blood ALAT activities was observed 3 hours after treatment with doses 1/20 LD_{50} and 1/10 LD_{50}, 24 hours after treatment with 1/5 LD_{50} and 48 hours – with 1/3 LD_{50}. ALAT activity remained elevated until the end of the study in the group treated at 1/2 LD_{50}.

The results from studies on activities of these two enzymes in rats [31] agree with our data. The simultaneous increase of both enzymes could be associated to enhanced synthesis and reduction of the conversion rates correlating to observed morphological changes in the liver parenchyma [23, 26, 32]. According to Gupta [15] the changes in blood ASAT and ALAT concentrations could result from vasodilation consequent to β_2-adrenoceptor effect of increased catecholamines level, established by Zaahkouk et al. [31]. Increased activity of aminotransferases after carbamate intoxication could be associated to increased permeability of cellular membranes due to ATP depletion in the cell. The observed considerable increase in serum ASAT and ALAT in all experimental groups regardless of the treatment dose was in line with activated liver function under the influence of carbamate pesticides and destruction of hepatocytes [31]. Applied orally, the carbamate first reaches the liver through the liver portal vein, stimulating the synthesis of ASAT and ALAT, demonstrated in vivo and in vitro by Kiran et al. [33]. According to the authors, the observed stimulation of ALAT activity results rather from from the interaction of the carbamate pesticide with the molecule of the enzyme than from interaction with tissues, which correlates with the hepatotoxic effect on liver parenchyma and other extrahepatal tissues. Transaminases (ASAT and ALAT) are a group of enzymes in the cytoplasm of living cells. The highest ALAT concentrations are detected in liver tissue, the lowest – in cardiac muscle and

relatively small amounts – in the brain, kidneys and blood serum. ASAT is observed at highest amounts in the liver, kidneys, the brain, skeletal and cardiac muscles. The increased activity of transaminases established in this experiment is indicative for a severe dehydration secondary to carbamate-induced stress – a mechanism, inducing elevation of serum transaminases' activity. Also, increased blood activity of transaminases is accepted as sign of tissue damage without damage of a specific organ. Damaged cells release transaminases into the blood circulation and other factors cold be also involved in this mechanism – altered permeability of cellular membrane, increased synthesis or reduced enzymatic degradation [31]. It is acknowledged that increased ASAT and ALAT levels are due to dystrophy and necrosis of hepatocytes, accompanied by damage of cellular walls and cytolysis and resulting flow of these mitochondrial enzymes into the circulation. ALAT is a more specific marker of liver damage as the enzyme increases only after destruction of cells from the liver parenchyma.

The analysis of changes in activity of lactate dehydrogenase (LDH) showed increased levels in all dogs treated with carbofuran by the 1st hour, proportionally to the dose of the toxic substance. The activities observed at that time are the peak LDH levels. Recovery of the levels of this parameter was observed by post treatment hour 5 (after a dose of 1/20 LD_{50}), hour 24 (at doses 1/10 LD_{50}, 1/5 LD_{50} and 1/3 LD_{50}) and hour 48 in dogs treated at 1/2 LD_{50}.

Our observations on increased blood LDH activity were in line with data of Zaahkouk et al. [31] and Rizos et al. [34], established in rats and men. According to Zaahkouk et al. [31] LDH enzymatic system plays a major role in the cellular glycolytic cycle for conservation of excessive energy under the form of pyruvate or lactate. This enzyme is contained in almost all tissues of the animal body and is released after tissue destruction, which in the view of authors is responsible for observed hyperglycaemia. Increased LDH activity parallelly to changes in ASAT and ALAT add to the hepatotoxicity of carbamate pesticides.

Data about the changes in blood activities of gamma glutamyltransferase (γ-GT) showed increased levels in all groups one hour after treatment. At that time, the peak γ-GT concentrations were recorded. The restoration of normal γ-GT activity in groups I, II and III, treated at 1/20 LD_{50}, 1/10 LD_{50} and 1/5 LD_{50} is observed by post treatment hour 7, while in groups IV and V (1/3 LD_{50} and 1/2 LD_{50}): by the 48th hour.

The observed increased blood γ-GT levels are supported by data of Rizos et al. [34], who reported a severalfold increase of this enzyme in a men after spontaneous intoxication with carbamate compounds. Gamma glutamyltransferase is encountered at highest concentrations in the liver, kidneys and the pancreas and is a sensitive marker for dystrophic damage in these organs. The mechanisms of increase in serum γ-GT concentrations are identical to those for ASAT and ALAT [31]. In quails treated with similar doses carbofuran, we failed to notice statistically significant changes in this parameter [30], which could be attributed to the species-specific sensitivity to the tested preparation.

The time course of alkaline phosphatase (ALP) activity showed increased levels in all experimental groups on post treatment hour 1. The values registered during that period are the highest ones. ALP activity was recovered by the 7th hour in group I (1/20 LD_{50}), by the 24th hour in groups II (1/10 LD_{50}) and III (1/5 LD_{50}) on hour 48 in group IV (1/3 LD_{50}) whereas in group V (1/2 LD_{50}) activities did not return to normal levels until the end of the study. It is acknowledged that alkaline phosphatase is found in cells of almost all organs, being at substantially higher concentrations in the liver. The observed elevation in blood ALP (about 6 times) was probably in relation to the hepatotoxic effect of carbamate pesticides and correlated with liver morphological changes [34]. Blood ALP changes in birds, rabbits and pigs [35, 36] treated with triazole pesticides were characterised with reduced ALP activity, therefore these data could assist the differential diagnosis of acute intoxications caused by triazole and carbamate pesticides.

Our studies on amylase activities showed about a twofold increase vs control values. These observations agreed with data reported by Moritz et al. [37], Rizos et al. [34], Goud et al. [38] and Tennakoon et al. [32] in spontaneous carbamate intoxications in humans. In available literature, there are no reports on blood amylase activity in animals after experimental carbamate intoxication. Carbamates inhibit both cholinesterase isoenzymes in the pancreas – acetylcholinesterase

and butyrylcholinesterase thus increasing the sensitivity of pancreas to acetylcholine effects. Cholinergic stimulation caused a secondary increase in pancreatic pressure and stimulates pancreatic secretion. In response to these events, pancreatitis develops in line with the increased blood amylase activity and dystrophic necrotic changes of pancreatic parenchyma [37, 38].

4. Conclusions

Our study showed that carbofuran exerted a strong inhibiting effect on acetylcholinesterase activity immediately after its application to experimental groups of dogs. A specific feature of intoxication with organocarbamate compounds is the spontaneous reactivation of AChE, which in our experiments was observed between the 4^{th} and 7^{th} hour after the challenge depending on the treatment dose. The analysis of results on the blood activity of aminotransferases in dogs treated with increasing doses of the carbamate pesticide carbofuran (1/20 LD_{50}, 1/10 LD_{50}, 1/5 LD_{50}, 1/3 LD_{50} and 1/2 LD_{50}) showed increased concentrations of ASAT, ALAT, γ-GT, LDH and ALP. The observed changes followed a stable trend towards elevation in all experimental groups regardless of the toxic dose. The simultaneous increase in blood ASAT (more than 8 times), ALAT (more than 6 times), γ-GT (more than 3 times), LDH (more than 4 times) and ALP (more than 6 times), as compared to control levels, confirmed the hepatotoxic effects of carbamate pesticides on liver parenchyma. The tested carbamate insecticide carbofuran exhibited a specific organotoxicity against the pancreas as seen from observed increase in blood amylase activities.

Acknowledgements

This study was funded by project No. №5/2005 and was supported by Faculty of Veterinary Medicine, Stara Zagora, Bulgaria.

References

1. Gupta, R. C., Carbofuran toxicity. Journal of Toxicology and Environmental Health, 1994, 43(4), 383-418.
2. Slaninova, A., Smutna, M., Modra, H. and Svobodova, Z., A review: oxidative stress in fish induced by pesticides. Neuroendocrinology Letters, 2009, 30(Suppl 1), 12-12.
3. Berny, P., Caloni, F., Croubels, S., Sachana, M., Vandenbroucke, V., Davanzo, F. and Guitart, R., Animal poisoning in Europe. Part 2: Companion animals. Veterinary Journal, 2010, 183, 255-259.
4. Tall, A., Ly, A. B., Nabeth, P., Sall, A. A., Sarr, F. D., Faye, J., Badiane, A., Diakhaby, G., Michel, R., Bar, N. A., Gomes Do Espirito Santo, M. E., Bazire, A., Marrama, L., Garnier, R., Simon, F., Rambourg, M. O. and Chevalier, B., Epidemiological investigation of death cases by pesticide poisoning. Journal of Agricultural Biotechnology and Sustainable Development, 2010, 2(4), 51-55.
5. Vlcek, V., Pohanka, M., Carbamate insecticides in the Czech Republic: Health and environmental impacts. Military Medical Science Letters, 2012, 81(1), 2-8.
6. Kervegant, M., Merigot, L., Glaizal, M., Schmitt, C., Tichadou, L. and de Haro, L., Paraquat poisonings in France during the European ban: experience of the Poison Control Center in Marseille. Journal of Medical Toxicology, 2013, 9(2), 144-147.
7. Motas-Gusman, M., Marla-Mojica, P., Romero, D., Martinez-Lopez, E. and Garcia-Fernandez, J., Intentional poisoning of animals in southeastern Spain: a review of the veterinary toxicology service from Murcia, Spain. Veterinary and Human Toxicolology, 2003, 45(1), 47-50.
8. Ogada, D. C., The power of poison: pesticide poisoning of Africa's wildlife. Annals of the New York Academy of Sciences, 2014, 1322(1), 1-20.
9. Ruiz-Suárez, N., Boada, L. D., Henríquez-Hernández, L. A., González-Moreo, F., Suárez-Pérez, A., Camacho, M., Zumbado, M., Almeida-González, M., Travieso-Aja, M. and Luzardo, O. P., Continued implication of the banned pesticides carbofuran and aldicarb in the poisoning of domestic and wild animals of the Canary Islands (Spain). Science of the Total Environment, 2015, 505, 1093-1099.
10. Wang, Y., Kruzik, P., Helsberg, A., Helsberg, I. and Rausch, W. D., Pesticide poisoning in domestic animals and livestock in Austria: A 6 years retrospective study. Forensic Science International, 2007, 169(2-3), 157-160.
11. Guitart, R., Croubels, S., Caloni, F., Sachana, M., Davanzo, F., Vandenbroucke, V. and Berny, P., Animal poisoning in Europe. Part 1: Farm livestock and poultry. Veterinary Journal, 2010a, 183(3), 249-254.
12. Guitart, R., Sachana, M., Caloni, F., Croubels, S., Vandenbroucke, V. and Berny, P., Animal poisoning in Europe. Part 3: Wildlife. Veterinary Journal, 2010b, 183(3), 260-265.
13. Novotný, L., Misík, J., Honzlová, A., Ondráček, P., Kuča, K., Vávra, O., Rachač, V. and Chloupek, P., Incidental poisoning of animals by carbamates in the Czech Republic. Journal of Applied Biomedicine, 2011, 9(3), 157-161.

14. Binev, R., Valchev, I., Russenov, A. and Nikolov, Y., Clinical toxicological investigations on acute carbofuran intoxication in dogs. Bulgarian Journal of Veterinary Medicine, 2014a, 17(1), 61-68.
15. Gupta, R. C., Brain regional heterogeneity and toxicological mechanisms of organophosphates and carbamates. Toxicology Mechanisms and Methods, 2004, 14, 103-143.
16. Martínez-Haro, M., Mateo, R., Guitart, R., Soler-Rodriguez, F., Perez-Lopez, M., Maria-Mojica, P. and Garcia-Fernandez, A. J., Relationship of the toxicity of pesticide formulations and their commercial restrictions with the frequency of animal poisonings. Ecotoxicology and Environmental Safety, 2008, 69, 396-402.
17. Modra, H., Svobodova, Z., Incidence of animal poisoning cases in the Czech Republic: current situation. Interdisciplinary Toxicology, 2009, 2(2), 48-51.
18. Aleksic, J., Mercep, D., Aleksic, Z. and Jovanovic, M., Dog poisoning with furadan 35-ST (carbamate insecticide). Veterinarski glasnik, 2011, 65, 277-285.
19. Wobeser, G., Bollinger, T., Leighton, F. A., Blakley, B. and Mineau, P., Secondary poisoning of eagles following intentional poisoning of coyotes with anticholinesterase pesticides in western Canada. Journal of Wildlife Diseases, 2004, 40(2), 163-172.
20. Kwon, K., Wee, H. and Kim, H., Pesticide poisoning events in wild birds in Korea from 1998 to 2002. Journal of Wildlife Diseases, 2004, 40(4), 737-740.
21. Berny, P., Pesticides and the intoxication of wild animals. Journal of Veterinary Pharmacology and Therapeutics, 2007, 30(2), 93-100.
22. Eddleston, M., Buckley, N. A., Eyer, P. and Dawson, A. H., Management of acute organophosphorus pesticide poisoning. Lancet, 2008, 371(9612), 597-607.
23. Lehel, J., Laczay, P., Déri, J., Darin, E. G. and Budai, P., Model study on the clinical signs and residue concentrations of sublethal carbofuran poisoning in birds. Journal of Wildlife Diseases, 2010, 46(4), 1274-1278.
24. Hovda, L. R., Hooser, S. B., Toxicology of newer pesticides for use in dogs and cats. Veterinary Clinics of North America: Small Animal Practice, 2002, 32(2), 455-467.
25. de Siqueira, A., Salvagni, F. A., Yoshida, A. S., Gonçalves-Jr, V., Calefi, A. S., Fukushima, A. R., Spinosa, H. S. and Maiorka, P. C., Poisoning of cats and dogs by the carbamate pesticides aldicarb and carbofuran. Research in Veterinary Science, 2015, 102, 142-149.
26. Padilla, S., Marshall, R. S., Hunter, D. L. and Lowit, A., Time course of cholinesterase inhibition in adult rats treated acutely with carbaryl, carbofuran, formetanate, methomyl, methiocarb, oxamyl or propoxur. Toxicology and Applied Pharmacology, 2007, 219(2-3), 202-209.
27. Ferré, D. M., Saldena, E. L., Albarracin, L., Neuilly, V. and Gorla, N. B., Butyrilcholinesterase inhibition and carbofuran analytical confirmation in two intoxicated dogs. Journal Revista Veterinaria, 2015, 26(1), 43-48.
28. Gupta, R. C., Milatovic, S., Dettbarn, W. D., Ascher, M. and Milatovic, D., Neuronal oxidative injury and dendritic damage induced by carbofuran: Protection by memantine. Toxicology and Applied Pharmacology, 2007, 219, 97-105.
29. Srebocan, E., Hrlec, G., Grabarevic, Z., Pompe-Gotal, J. and Sabocanec, R., Poisoning with acetylcholinesterase inhibitors in dogs: two case reports. Veterinarni Medicina, 2003, 48, 175-176.
30. Binev, R., Valchev, I., Stoyanchev, K., Mihaylov R. and Nikolov, Y., Changes in blood enzyme activities after experimental acute intoxication of quails (Coturnix coturnix) with the carbamate insecticide carbofuran. Bulgarian Journal of Veterinary Medicine, 2014b, 17(4), 331-337.
31. Zaahkouk, S. A. M., Helal, E. G. E., Abd-Rabo, T. E. I., Somaia, Z. A. R., Carbamate toxicity and rotective effect of vit. A and vit. E on some biochemical aspects of male albino rats. The Egyptian Journal of Hospital Medicine, 2000, 1, 60-77.
32. Tennakoon, D. A. S., Karunarathna, W. D. V. and Udugampala, U. S. S., Carbofuran concentrations in blood, bile and tissues in fatal cases of homicide and suicide. Forensic Science International, 2013, 227(1-3), 106-110.
33. Kiran, R., Bansal, M. and Banal, R. C., Effect of carbamates on some enzymes of rat liver and kidney. Pesticides, 1988, 22(3), 8-10.
34. Rizos, E., Liberopoulos, E., Kosta, P., Efremidis, S. and Elisaf, M., Carbofuran- induced acute pancreatitis. Journal of Pancreas, 2004, 5(1), 44-47.
35. Binev, R., Clinical and experimental studies on acute intoxication with the triazole fungicide Diniconazole (Sumi 8 2WP) in rabbits. Studies upon activity some enzymes. Bulgarian Journal of Veterinary Medicine, 2002, 5(4), 253-259.
36. Binev, R., Simeonov, R., Todorov R. and Nikolov, Y., Pathomorphological studies following experimental acute intoxication with the triazole fungicide triticonazole in pigs. Bulgarian Journal of Veterinary Medicine, 2005, 8(3), 193-198.
37. Moritz, F., Droy, J.M., Dutheil, G., Melki, J., Bonmarchand, G. and Leroy, J.,Acute pancreatitis after carbamate insecticide intoxication. Intensive Care Medicine, 1994, 20(1), 49-50.
38. Goud, M., Nayal, B., Deepa, K., Devi, O. S., Devaki, R. N. and Anitha, M., A case of acute pancreatitis with occupational exposure to organophosphorus compound. Toxicology Internacional, 2012, 19(2), 223-224.