

CLINICAL AND EXPERIMENTAL STUDIES ON ACUTE
INTOXICATION WITH THE TRIAZOLE FUNGICIDE
DINICONAZOLE (SUMI 8 2WP) IN RABBITS.
III. STUDIES UPON THE ACTIVITY OF SOME ENZYMES

R. BINEV

Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary

Binev, R., 2002. Clinical and experimental studies on acute intoxication with the triazole fungicide diniconazole (Sumi 8 2WP) in rabbits. III. Studies upon the activity of some enzymes. *Bulg. J. Vet. Med.*, **5**, No 4, 253–259.

Experiments for evaluation of the toxic effect of the triazole fungicide diniconazole (Sumi 8 2WP) on rabbits were carried out following up the changes in blood activities of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), lactate dehydrogenase (LDH) and cholinesterase (ChE). The studies were performed in one control and three experimental groups, treated orally via a gastic tube with increasing doses of diniconazole (0.1LD₅₀, 0.5LD₅₀ and LD₅₀ – oral doses for rats).

Blood for analysis of enzyme activities was sampled at hours –48, –24 and 0 prior to the treatment and hours 2, 4, 6, 8, 10, 12, 24, 48 and 72 after the treatment from all animals.

It was observed that the triazole fungicide diniconazole had a toxic effect manifested by increased ASAT, ALAT and LDH activities, decreased AP activity and no changes in ChE.

Key words: alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, cholinesterase, diniconazole, lactate dehydrogenase, Sumi 8 2WP, triazole fungicide

INTRODUCTION

It is known that the toxic effect of triazole fungicides is accompanied with changes in activities of several enzymes. Data in this respect were reported about the effect of cyproconazole, flusilazole, 1,2,4-triazole and propiconazole in mice, rats and humans (Machera, 1995); Baytan (triadimenol) – in cattle (Markelov and Shormanov, 1994) and hens (Binev, 2000d); of Tilt (propiconazole) – in cats following intratracheal administration (Kurumbaev et al., 1996); of dialkylimidazole – in mice (Sivertsen and Muller, 1999) and rats (Patel et al., 1998a; Patel et al. 1998b). Those studies evidenced that depending on the amount of the toxic sub-

stance, the blood activities of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and lactate dehydrogenase (LDH) increased while data about the levels of alkaline phosphatase (AP) and cholinesterase (ChE) were contradictory.

In previous studies of ours upon the acute intoxication with the triazole fungicide diniconazole in rabbits the changes in the clinical status (hypothermia, tachycardia, polypnea) and some haematological changes (oligochromemia, erythropenia, neutrophilia, lymphocytopenia and slower erythrocyte sedimentation rates) were described (Binev, 2001). Hens,

treated orally with diniconazole at 0.064 g/kg (0.1LD₅₀ oral dose for rats); 0.320 g/kg (0.5LD₅₀ oral dose for rats) and 0.639 g/kg (LD₅₀ oral dose for rats), showed no anticholinesterase effect (Binev, 2000a; 2000b; 2000c). However, there are not any other literature data about the toxic influence of diniconazole in domestic animals, including its effects upon enzyme activity.

Our study aimed to determine the influence of diniconazole upon the activity of foresaid enzymes and its probable relationship with the clinical, haematological and biochemical changes in rabbits (Binev, 2001) and hens (Binev, 2000a; 2000b; 2000c).

MATERIALS AND METHODS

Animals

The studies were performed with 22 rabbits from both genders, California and White New Zealand breed, aged 6-8 months and weighing 2.8–3.2 kg. The animals were put in individual cages 30 days prior to the beginning of the experiments under standard hygienic conditions. The rabbits received treatments against endoparasites with piperazine sulfate (Piperatrin, Vetprom, Bulgaria) and against ectoparasites with 0.1% cypermethrin solution (Ectomin, Ciba-Geigy, Switzerland). The daily ration of each animal consisted of 0.1 kg combined concentrate and 0.2 kg alfalfa hay with free access to tap water.

Drugs and treatments

An acute intoxication was provoked with the commercial preparation Sumi 8 2WP containing 2% diniconazole and 98% inert ingredients (including surfactant and tetrahydrofurfuryl alcohol) (Sumitomo Chemical, Japan). It was administered to experimental animals once, internally,

using a gastric tube.

Experimental design

The rabbits were divided into 4 groups – one control (n=4) and three experimental groups (n=6 each). The control group was left untreated while the experimental ones received diniconazole in doses as followed:

- group I: 0.064 g/kg or 3.2 ml/kg of Sumi 8 2WP (= 0.1 oral LD₅₀ for rats);
- group II: 0.320 g/kg or 16 ml/kg of Sumi 8 2WP (= 0.5 oral LD₅₀ for rats);
- group III: 0.639 g/kg or 32 ml/kg of Sumi 8 2WP (= oral LD₅₀ for rats).

Blood from *v. jugularis* or *v. femoralis* was sampled three consecutive days prior to the treatment (hours -48, -24 and 0) and 2, 4, 6, 8, 10, 12, 24, 48 and 72 hours thereafter.

Analysis of samples

The activities of ASAT, ALAT, AP and LDH were assessed in blood serum by a biochemical analyser (Cobas Mira plus) with kits of Human Diagnostica, Germany. The methods are based on the optical test of Warburg, measuring the maximum enzyme activity in optimal conditions. Transaminase activities (ASAT, ALAT) were determined by a kinetic method recommended by the International Federation of Clinical Chemistry in 20 µl serum at 37 °C. AP activity was measured by an optimized and standardized method recommended by the German Society of Clinical Chemistry in 20 µl serum at 37 °C. LDH was determined by the modified method of the Scandinavian Committee of Enzymes (SCE) whereas serum ChE – by Ellmann's reactive, measuring the amount of formed choline by the method of Angelov et al. (1999).

Statistical analysis

Data are presented as mean ± SEM. The statistical significance of results was de-

terminated using the Student t-test at a level of significance of $p \leq 0.05$. Comparisons between control values and the values in experimental groups for each experimental interval were performed. All p-values, mentioned thereafter are referring to those differences.

RESULTS

All animals from group III died by hour 4 after the treatment (the data for clinical and haematological changes are presented in a previous report) (Binev, 2001).

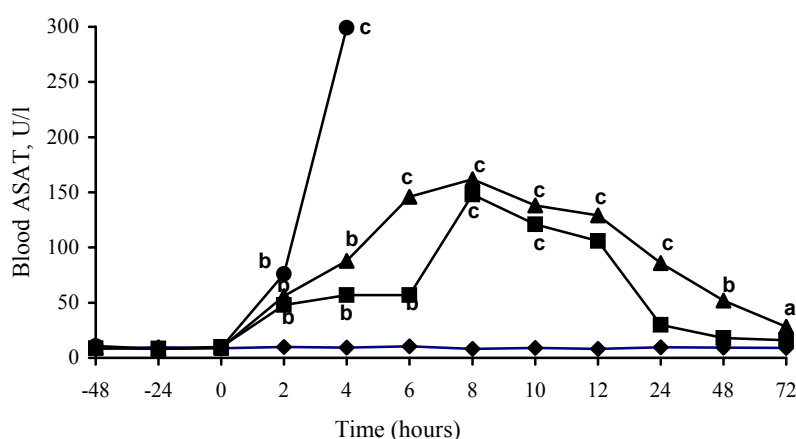


Fig. 1. Changes in blood ASAT activities in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ♦ control group; ■ group I; ▲ group II; ● group III.

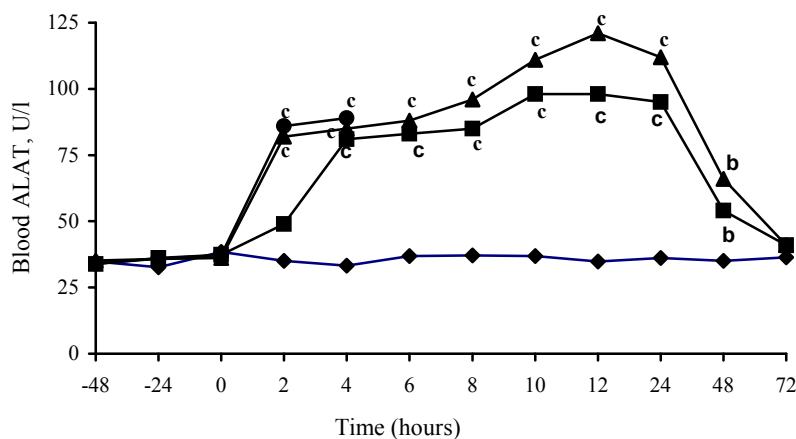


Fig. 2. Changes in blood ALAT activities in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ♦ control group; ■ group I; ▲ group II; ● group III.

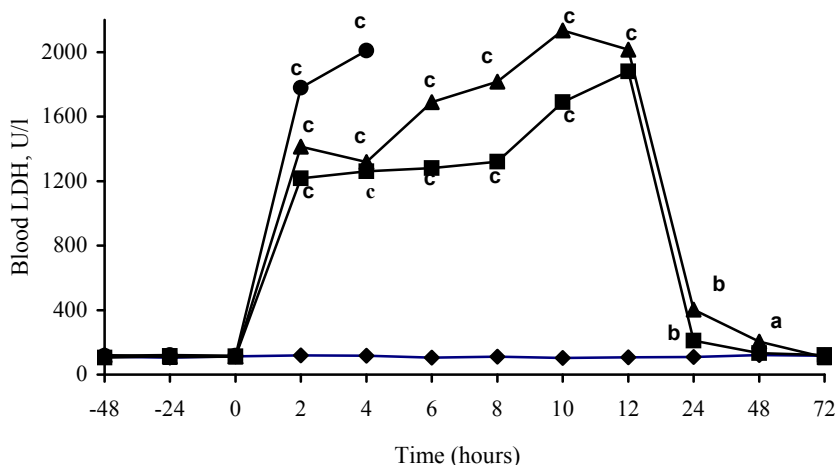


Fig. 3. Changes in blood LDH activities in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ♦ control group; ■ group I; ▲ group II; ● group III.

ASAT activity (Fig. 1) in control rabbits varied between 8.2 ± 0.7 U/l and 9.6 ± 0.8 U/l prior to and between 8.2 ± 0.7 U/l and 10.4 ± 0.9 U/l after the treatment, respectively. In all experimental groups, serum ASAT increased 2 hours after diniconazole administration. In groups I and II the peak activities were reached by hour 8 (148 ± 8.2 U/l and 162 ± 9.8 U/l respectively). In group III, enzyme activity was 299 ± 18.7 U/l by the 4th hour, when death occurred. At hour 48, ASAT levels were similar to initial ones in group I. In group II ASAT remained elevated even by hour 72 (28 ± 3.6 U/l).

Post treatment ALAT activities (Fig. 2) were also increased compared to control rabbits, where ALAT levels ranged between 32.6 ± 3.1 U/l prior to and 33.3 ± 3.1 U/l after diniconazole challenge. In group I, ALAT activity reached 81 ± 6.2 U/l ($p < 0.01$) by hour 4; in group II – 82 ± 6.8 U/l ($p < 0.01$) by hour 2. The peak ac-

tivities were by hour 10–12 in group I (98.2 ± 7.6 U/l); by hour 12 in group II (121.2 ± 11.4 U/l) and by hour 4 (89.7 ± 6.1 U/l) in group III. Seventy two hours after the treatment, ALAT levels were close to initial ones.

LDH dynamics (Fig. 3) was similar to that of ASAT and ALAT. Compared to the control group, where LDH was 109 ± 9.2 U/l – 114 ± 9.8 U/l prior to and 104 ± 8.8 U/l – 119 ± 10.2 U/l following diniconazole treatment, significantly elevated activities were observed by post treatment hour 2 in all treated groups ($p < 0.001$). Peak activities were detected by hour 12 in group I (1880 ± 104 U/l), by hour 10 (2134 ± 162 U/l) in group II and by hour 4 (2010 ± 126 U/l) in group III. LDH levels returned to the normal range 48 and 72 hours after the challenge in group I and II respectively.

AP activities in controls varied between 562 ± 48 U/l and 620 ± 64 U/l be-

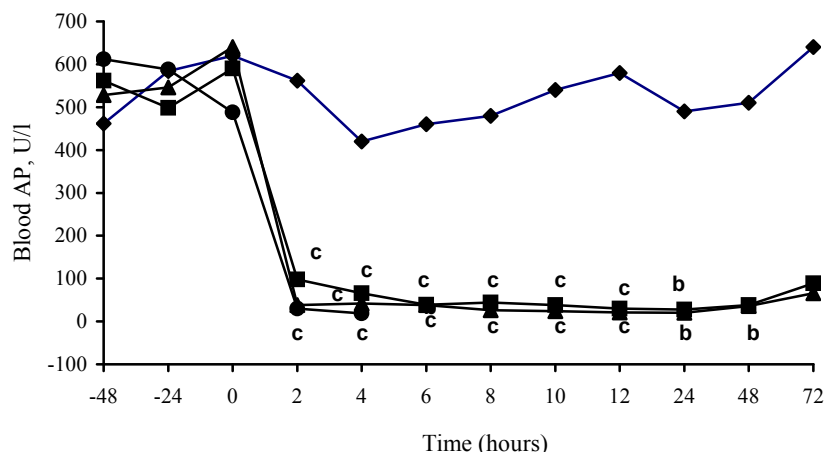


Fig. 4. Changes in blood AP activities in rabbits - untreated (controls) and treated orally with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ♦ control group; ■ group I; ▲ group II; ● group III.

fore the treatment and between 420 ± 46 U/l and 640 ± 68 U/l afterwards. In groups I and II, this parameter decreased from the beginning to the end of the experimental period with lowest activities by post treatment hour 24 – 28.2 ± 1.4 U/l and 20.1 ± 1.8 U/l respectively. In group III, AP levels by hour 4 were 19 ± 1.1 U/l.

The changes in ChE activity in treated animals were not statistically significant compared to controls.

DISCUSSION

The experimental intoxication with the triazole fungicide diniconazole in rabbits was accompanied by changes in the activities of some of studied enzymes (ASAT, ALAT, LDH and AP). The increasing toxic doses of 0.064 g/kg ($= 0.1$ oral LD_{50} for rats); 0.320 g/kg ($= 0.5$ oral LD_{50} for rats) and 0.639 g/kg ($=$ oral LD_{50} for rats) resulted in increased activities of aminotransferases and LDH. A similar influ-

ence was reported by Machera (1995) in mice, rats and humans treated with ciproconazole, flusilazole, 1, 2, 4-triazole and propiconazole; by Patel et al. (1998a) in rats, by Ronis et al. (1998) in Japanese quails and *in vitro* under the influence of 1,2,4-triazole (Celic et al., 1996).

The simultaneous increase in ASAT, ALAT and LDH could be explained by the toxic influence of diniconazole upon liver parenchyma. A diniconazole-mediated impaired liver function manifested by bilirubinaemia were observed by us in hens (Binev, 2000b). The data from pathomorphological studies in cattle (Markelov and Shormanov, 1994) following treatment with Baytan (triadimenol) and rats (Patel et al. 1999) as well as the data observed after 1,2,4-triazole challenge are in agreement with our observations. According to Markelov and Shormanov (1994), Patel et al. (1999) and Filipov and Lawrence (2001), the increased enzyme activity was caused by liver dystrophy. In the present study, the

forementioned changes were manifested by an over 20-fold increase in LDH levels, over 15-fold increase in ASAT levels and over 4-fold increase in ALAT activity (compared to controls) with a De Ritis index (ASAT/ALAT) >1.

The decreased AP activity (over 20-fold compared to controls) was observed also in mice, rats and humans treated with cyproconazole, flusilazole, 1,2,4-triazole and propiconazole (Machera, 1995), in Japanese quails (Ronis et al., 1998) and rats (Patel et al., 1998b) following 1,2,4-triazole treatment. Most probably, those changes are related to the antithyroid effect of triazole fungicides, shown for 3-amino-1,2,4-triazole (Mayberry, 1968), cyproconazole, flusilazole and propiconazole (Machera, 1995) and 1,2,4-triazole (Patel et al., 1998a) as well as by the pathomorphological alterations in bovine thyroid gland observed after Baytan (triadimenol) challenge (Markelov and Shormanov, 1994).

The lack of statistically significant changes in ChE activity showed that diniconazole had no anticholinesterase effect in rabbits. The same finding was reported in hens, treated with the same preparation (Binev, 2000b) as well as with Baytan (triadimenol) (Binev, 2000d).

In conclusion it could be stated that the intoxication with the triazole fungicide diniconazole (contained in the composition of the preparation Sumi 8 2WP) used in toxic doses ($= 0.1 \times LD_{50}$; $0.5 \times LD_{50}$ and the oral LD_{50} for rats) in rabbits was manifested by increased ASAT, ALAT and LDH activities, decreased AP activity and unchanged ChE activity.

The influence of diniconazole upon the enzyme activity could be evaluated as important and have to be considered during the elucidation of its toxicokinetics and toxicodynamics as well as during the

diagnostics, treatment and prophylaxis of acute triazole fungicide intoxication.

REFERENCES

- Angelov, G., N. Ibrishimov and S. Milashki (1999). Clinical Laboratory Studies in Veterinary Medicine, "Prof. Marin Drinov", Sofia (Bg).
- Binev, R., 2000a. Clinical and experimental studies on the acute intoxication with the triazole fungicide diniconazole in birds. I. Clinical and paraclinical studies. *Veterinary Medicine (Sofia)*, No 1, 44–48 (Bg).
- Binev, R., 2000b. Clinical and experimental studies on acute intoxication with the triazole fungicide diniconazole in birds. II. Biochemical studies. *Veterinary Medicine (Sofia)*, No 1, 49–52 (Bg).
- Binev, R., 2000c. Clinical and experimental studies on the preparation Sumi 8 2WP in hens. In: Proceedings of the Scientific Conference "Achievements in the Field of Agricultural and Social Sciences", Union of Scientists in Bulgaria, Branch Stara Zagora, Part 1, 169–174 (Bg).
- Binev, R., 2000d. Clinical and haematological studies on the acute poisoning with the triazole fungicide Baytan (triadimenol) in hens. In: Proceedings of the Scientific Conference "Ecology and Health", House of Science and Technology, Academic Publisher of the Agricultural University Plovdiv, No 1, 131–134 (Bg).
- Binev, R., 2001. Clinical and experimental studies on acute poisoning with the triazole fungicide diniconazole (Sumi 8 2WP) in rabbits. I. Clinical and haematological studies. *Bulgarian*

- Journal of Veterinary Medicine*, **4**, No 2, 103–114.
- Celic, I., H. Camas, O. Arslan, E. Yegin and I. Kutrevioglu, 1996. The effects of some pesticides on the activity of liver and erythrocyte enzymes (in vitro). *Journal of Environmental Science and Health*, **7-A**, 1645–1649.
- Filipov, N. and D. Lawrence, 2001. Developmental toxicity of a triazole fungicide: consideration of interorgan communication. *Toxicology Science*, **62**, No 2, 339–352.
- Kurumbaev, Y. K., S. O. Kulyakova, N. V. Sanotskaya and S. O. Aleynikov, 1996. Pathomorphological study of lungs in feline pneumonia model. *Bulletin of Experimental Biology and Medicine*, No 1, 103–104 (Ru).
- Machera, K., 1995. The studies on the effects of triazole fungicides: cyproconazole, flusilazole, 1,2,4-triazole and propiconazole. *Bulletin of Environmental Contamination Toxicology*, **54**, 363–369.
- Markelov, M. Y. and V. K. Shormanov, 1994. Distribution of the pesticide Baytan and its primary metabolite (DTChB) in warm-blooded animals. *Pharmacia*, No 6, 16–19 (Ru).
- Mayberry, W. E., 1968. Antithyroid effects of 3-amino-1,2,4-triazole. *Experimental Biology and Medicine*, **129**, 551–556.
- Patel, Y., H. S. Kushwah, A. Kushwah and M. A. Quardi, 1998a. *In vitro* action of pesticides mixture on certain metabolic enzymes in rats. *Indian Veterinary Journal*, **75**, No 7, 600–603.
- Patel, Y., H. S. Kushwah, A. Kushwah and M. A. Quardi, 1998b. Effect of chronic pesticides mixture toxicity on some enzymes in rats. *Indian Veterinary Journal*, **75**, No 8, 698–700.
- Patel, Y., H. S. Kushwah, A. Kushwah and M. R. Malik, 1999. Histopathological changes induced by pesticide in rats. *Indian Veterinary Journal*, **76**, No 10, 930–931.
- Ronis, N. J., M. Celander and T. M. Badger, 1998. Cytochrome P450 enzymes in the kidney of Bobwhite quail (*Colinus virginianus*): induction and inhibition by ergosterol biosynthesis inhibiting fungicides. *Comparative Biochemistry and Physiology C – Pharmacology, Toxicology & Endocrinology*, **121**, No 1–3, 221–229.
- Sivertsen, T. and L. Muller, 1999. Ammoniated forage poisoning: acute toxicity of newly identified dialkylimidazoles to inbred mice. *Veterinary and Human Toxicology*, **41**, No 6, 363–368.

Paper received 05.11.2001; accepted for publication 03.07. 2002

Correspondence:

Dr. R. Binev
Dept. of Internal Medicine,
Faculty of Veterinary Medicine,
Trakia University,
6000 Stara Zagora, Bulgaria
e-mail: binew@abv.bg

