

CLINICAL AND EXPERIMENTAL STUDIES ON ACUTE
INTOXICATION WITH THE TRIAZOLE FUNGICIDE
DINICONAZOLE (SUMI 8 2WP) IN RABBITS.
I. CLINICAL AND HAEMATOLOGICAL STUDIES

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Summary

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Experiments for evaluation of the toxic effect of the triazole fungicide diniconazole (Sumi 8 2WP) on rabbits were carried out through following up the changes in the clinical status (body temperature, heart and respiratory rates) and some haematological indices (haemoglobin concentration, red blood cell counts, total and differential white blood cell counts and erythrocyte sedimentation rates). The studies were performed upon one control and three experimental groups, treated orally via a gastric tube with increasing doses of the preparation (0.1LD₅₀, 0.5LD₅₀ and LD₅₀ - oral doses for rats).

All animals were clinically examined and blood for analysis 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.

It was found out that the tested triazole fungicide exerted a toxic effect manifested by hypothermia, tachycardia, polypnea, oligochromemia, erythropenia, neutrophilia, lymphocytopenia and slower erythrocyte sedimentation rates. Those changes were the most expressed between post treatment hours 2 and 24 and afterwards the values returned to initial values.

Key words: triazole fungicide, diniconazole (Sumi 8 2WP), rabbits, clinical and haematological parameters

INTRODUCTION

At present, fungicides are about 30% of all pesticides used. Their extensive utilization is motivated by the broad distribution of fungal diseases on agricultural cultures including cereals and leguminous plants. From the middle of the 90-ties, the fight against these diseases is performed exclusively with triazole fungicides – hexaconazole, cyproconazole, triadimenole, bilocsazole, diclobutrazole, tebuconazole, difenconazole, diniconazole, propiconazole etc. (Sengalevich et al., 1998). They have completely replaced preparations containing lead and copper (Tasheva,

1996).

Diniconazole is a triazole fungicide, intended for decontamination of seeds from cereals (wheat, barley etc.) and leguminous plants. It kills the fungal agents including those in the soil during the period of vegetation. Its mechanism of influence is to impair the metabolism of fungal cells via inhibition of ergosterol synthesis and damage of cellular membranes, resulting in termination of their development and death (Iliev, 1996). The fungicide has a low acute toxicity. The oral LD₅₀ for rats is 639 mg/kg, for quails – 1490 mg/kg, for

ducks – 2000 mg/kg. It is highly toxic for fish – LC_{50} (96 h) is 4 mg/l. The cutaneous LD_{50} in rats is over 5000 mg/kg while the inhalatory LC_{50} (4 h) - over 2770 mg/m^3 (Sengalevich et al., 1998).

The toxic effects in humans following contact with the skin, mucosas and the upper respiratory tract are difficult breathing, salivation, rhinitis, lacrimation, cutaneous and ophtalmic erythema, corneal opacity, pruritus, eczema, dermatitis, tracheobronchitis, bronchitis and bronchopneumonia. One hour after ingestion, depression, ataxia, nausea, salivaion, difficult breathing, hypothermia, haemoglobinemia, decreased haematocrit values and red blood cell (RBC) counts are reported (Machera, 1995). Our studies in hens treated internally with diniconazole at dose rates of 0.064 g/kg, 0.320 g/kg and 0.639 g/kg (corresponding to 0.1 LD_{50} , 0.5 LD_{50} and LD_{50} for oral administration in albino rats, respectively) showed a variety of clinical (hypothermia, polypnea, paresis of wings and limbs, clonic spasms of cervical muscles and termination of the laying capacity), haematological (oligochromaemia and oligocythaemia) (Binev, 2000a) and biochemical (hyperglycaemia, lacticidaemia, hyperalbuminaemia, hypo- α_1 - and γ -globulinaemia, uremia, hypercholesterinaemia, hypercalcaemia and hyperphosphataemia). The study of acetylcholinesterase did not reveal an anticholinesterase activity in hens (Binev, 2000b). In addition to those results, other literature data for the effect of diniconazole in domestic animals, including rabbits, were not found.

There are however reports about some changes in the clinical status and the blood indices in laboratory animals (mice and rats) under the influence of other triazole fungicides (cyproconazole, flusilazole, propiconazole) (Shandrenko and

Dmitrenko, 1996; Patel et al., 1998). Changes occurring in humans (Machera, 1995), in cattle using Baytan (triadimenol) (Markelov and Shormanov, 1994) and in cats after intratracheal administration of Tilt (propiconazole) (Kurumbaev et al., 1996) have also been studied. There was a dose-dependent effect manifested by accelerated heart and respiratory rates and decreased body temperature in cats, cattle, rats, mice and humans. The haematological changes consisted in decreased haemoglobin concentrations, decreased RBC counts; increased or normal white blood cell (WBC) counts with neutrophilia and lymphocytopenia and lower erythrocyte sedimentation rates (ESR).

All those data motivated the performance of complex experimental studies with diniconazole to establish the changes in the clinical and haematological status during the acute intoxication in rabbits in order to render easy the diagnostics, treatment and prophylaxis of triazole fungicide intoxications.

MATERIALS AND METHODS

Animals

The studies were performed with 22 rabbits from both genders, California and White New Zealand breeds, 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 in standard hygienic conditions. The rabbits also 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 received diniconazole in doses as followed:

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

Assessment of toxicity

The clinical status was followed up in 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, registering the rectal body temperature (BT), the heart and respiratory rates (HR and RR), the colour of visible mucosas, general condition, locomotor activity, sensory status, urination, defecation etc. using routine methods. Simultaneously, blood was sampled from *v. jugularis* or *v. femoralis* for determination of haemoglobin (Hb), RBC and total WBC counts with an automated haemoanalyser (Serono-System 150, USA), the differential WBC counts – via counting on a blood smear;

the ESR at hour 24 – according to Westergreen (Angelov et al., 1999).

Statistical analysis

Data are presented as mean $\pm$ SEM. The statistical significance of results was determined using the Student t-test at a level of significance of $p < 0.05$. Comparisons between control values (using an average for the whole experimental period) and the values in experimental groups for each period of the study were performed. All p-values, mentioned thereafter are referring to those differences.

RESULTS

The BT in all groups did not show any significant deviations in the period prior to the treatment (Fig. 1). In the control group, it was averagely 38.4 ± 0.2 °C during the whole period of the study. After the administration of increasing doses of the preparation, BT decreased. In group I, it reached 38.1 ± 0.1 °C by hour 2 ($p < 0.05$) with lowest values being detected between post treatment hours 4 and 6 (37.2 ± 0.1 and 37.0 ± 0.1 °C, respectively; $p < 0.001$). In the interval between hours 10 and 12 BT returned to the normal range. The BT decrease in rabbits from group II was to 37.8 ± 0.1 °C by hour 2 ($p < 0.001$) with a minimum at hour 6 (36.8 ± 0.1 °C; $p < 0.001$). After the post treatment hour 24, BT restored its initial values. In the third experimental group, the BT decreased to 37.2 ± 0.1 °C at hour 2 ($p < 0.001$) and 35.8 ± 0.2 °C at hour 4 ($p < 0.001$). Afterwards, all animals from group III died.

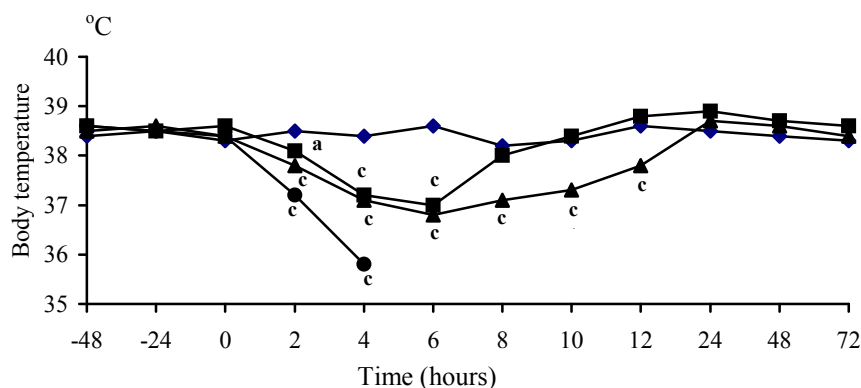


Fig. 1. Changes in body temperature in rabbits - untreated (controls) and treated with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^c $p < 0.001$.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

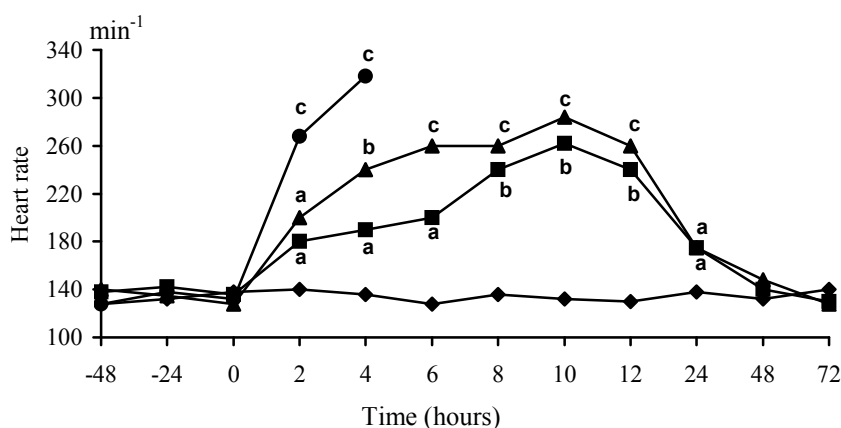


Fig. 2. Changes in heart rate in rabbits - untreated (controls) and treated with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p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

The HR (Fig. 2) in the control group was $134 \pm 11 \text{ min}^{-1}$. Under the influence of diniconazole, it was accelerated in animals from groups I and II reaching a peak

at hour 10: 262 ± 22 ($p < 0.01$) and 284 ± 23 ($p < 0.001$) min^{-1} respectively. The peak values in the third group were recorded by hour 2 ($268 \pm 18 \text{ min}^{-1}$; $p < 0.001$) and

immediately prior to the lethal issue (hour 4, $318 \pm 28 \text{ min}^{-1}$; $p < 0.001$). Between hours 24 and 48 of the course of intoxication, heart rates normalized.

The respiratory rates (Fig. 3) in untreated animals were similar during the whole period of the study. In experimental animals, they changed like heart rates. Significant differences compared to controls were observed within hours 2 and 8 following treatment. The peak average RR was by hour 6 for group I ($82 \pm 6 \text{ min}^{-1}$; $p < 0.01$) and group II ($86 \pm 6 \text{ min}^{-1}$; $p < 0.01$). The RR values returned to normal after the 24th hour of the study. In the third group, RR reached $104 \pm 9 \text{ min}^{-1}$ by hour 4 ($p < 0.01$).

The general condition and behaviour of experimental animals during the first 2 hours of the intoxication were not changed. In the next interval they refused food and water, their conjunctivae became pale and the cornea - opaque. There was a

lacrimation, salivation and difficult breathing. In group II together with all those signs, tremor and spasms of the skeletal muscles appeared. The clinical symptoms in group I passed after hour 6 while in the second group - after the 8th hour. In the third experimental group apart the forementioned signs, convulsions, clonic spasms of the cervical musculature manifested by rhythmic elevation of the head were observed in the period between hours 4-6, i.e. immediately prior to the lethal issue.

The Hb content in the control group was about $138.8 \pm 6.8 \text{ g/l}$. Following the treatment it decreased and at hour 2 reached $107.1 \pm 5.4 \text{ g/l}$ ($p < 0.05$) for group I, $94.3 \pm 6.2 \text{ g/l}$ ($p < 0.01$) for group II and $90.2 \pm 5.8 \text{ g/l}$ ($p < 0.001$) for group III (Fig. 4). The oligochromemia was the most expressed at hour 8 in group I ($64 \pm 5.3 \text{ g/l}$) and hour 24 ($52 \pm 4.7 \text{ g/l}$) in group II. In the interval between hours 48-

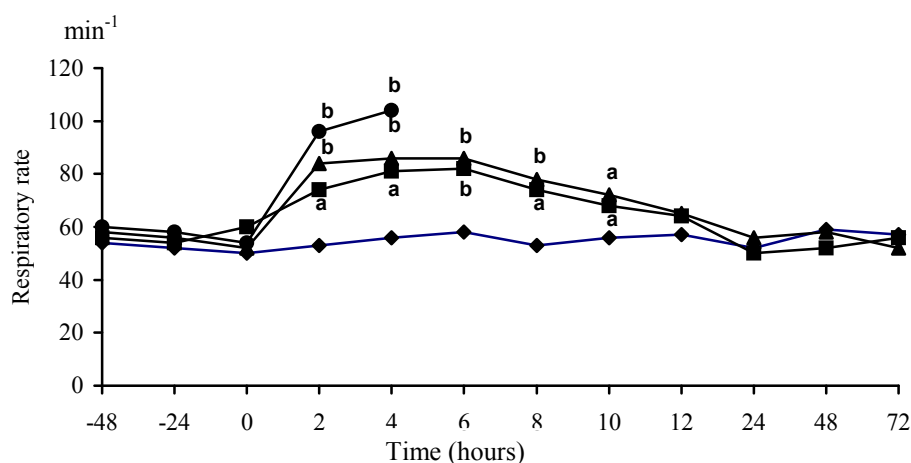


Fig. 3. Changes in respiratory rate in rabbits - untreated (controls) and treated with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$.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

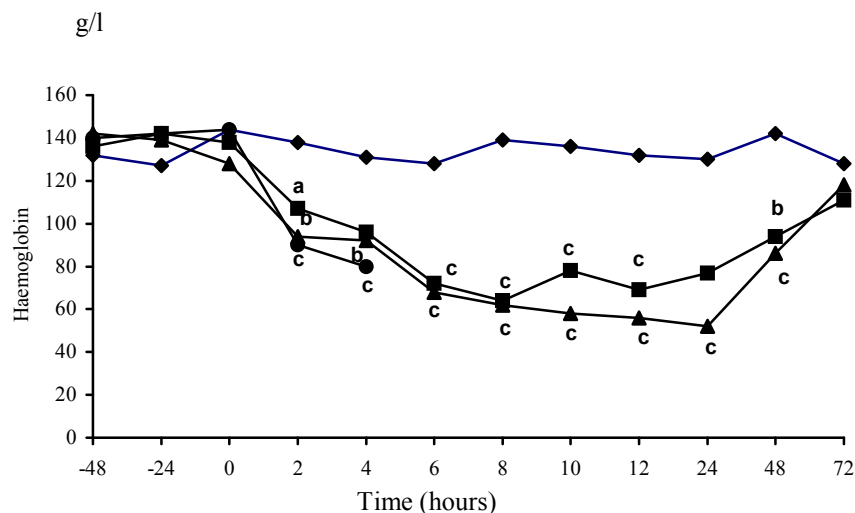


Fig. 4. Changes in haemoglobin content in rabbits - untreated (controls) and treated with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$;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

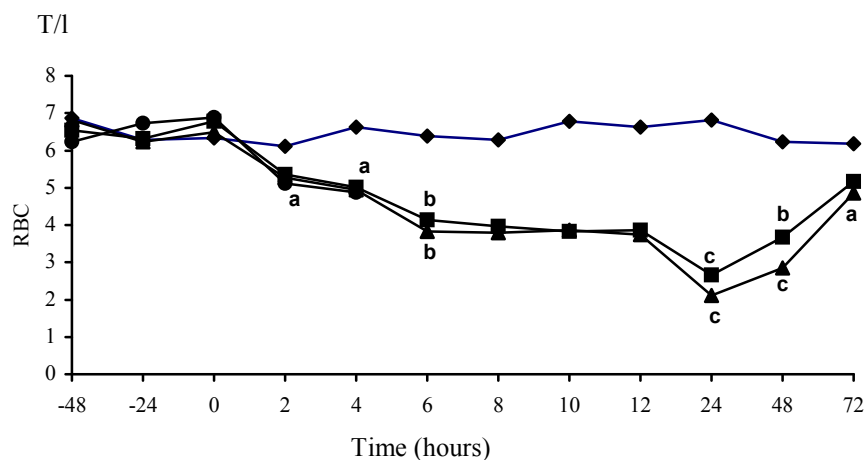


Fig. 5. Changes in RBC counts in rabbits - untreated (controls) and treated with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ◆ control group; ■ group I; ▴ group II; ● group III.

72 Hb was still lower than the initial values but with a tendency towards normalization. Before the death of the animals

from group III, Hb levels were 78.8 ± 6.2 g/l ($p < 0.001$).

The RBC counts in control rabbits were about 6.54 ± 0.32 T/l (Fig. 5). After the 4th hour, they decreased in groups I and II with lowest counts at hour 24 - 2.66 ± 0.22 T/l for the former and 2.11 ± 0.19

statistically insignificant elevation. There were however changes in the percentages of leukocyte classes. The segmented neutrophils (Fig. 6) in the control group were $34.6 \pm 3.8\%$. By hour 4 they increased in

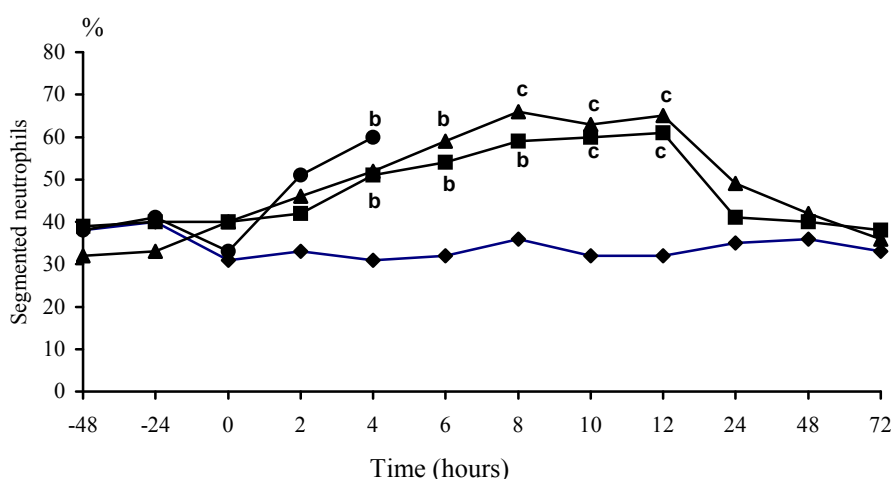


Fig. 6. Changes in segmented neutrophils in rabbits - untreated (controls) and treated with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ◆ control group; ■ group I; ▲ group II; ● group III.

T/l for the latter ($p < 0.001$). At hour 72, RBC counts were in the normal range for group I and yet lower than normal in group II although with a tendency towards normalization. At hour 4, RBC counts reached 4.88 ± 0.31 T/l in the third experimental group ($p < 0.01$).

The total WBC counts in experimental groups I and II were close to those in untreated animals. In group III there was a

all three experimental groups: $51.4 \pm 4.2\%$ ($p < 0.01$) for group I, $51.8 \pm 4.9\%$ ($p < 0.01$) for group II ($p < 0.01$) and $62.3 \pm 5.8\%$ ($p < 0.01$) for group III. The peak values were observed at hour 12 for group I ($65.3 \pm 5.8\%$; $p < 0.001$) and hour 8 for group II ($69.6 \pm 5.8\%$; $p < 0.001$). The increased percentages were preserved up to the 12th hour. At hour 48 segmented neutrophils returned to initial percentages.

Lymphocytes in the control group were averagely $55.4 \pm 4.2\%$ (Fig. 7). Following the treatment they decreased espe-

cially between hours 4 and 24, being the lowest at hour 10 for group I and hour 12 for group II ($22.33 \pm 2.8\%$ and $21.1 \pm$

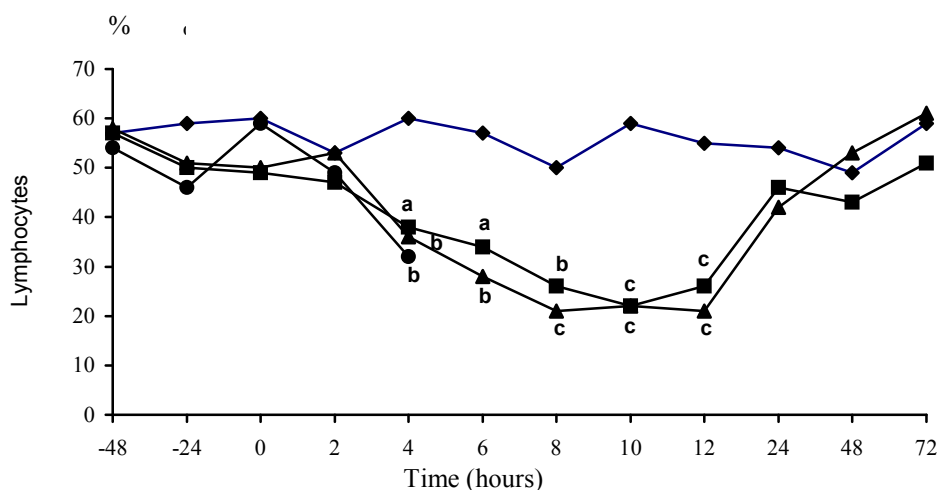


Fig. 7. Changes in lymphocyte percentage in rabbits - untreated (controls) and treated with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ♦ control group; ■ group I; ▴ group II; ● group III.

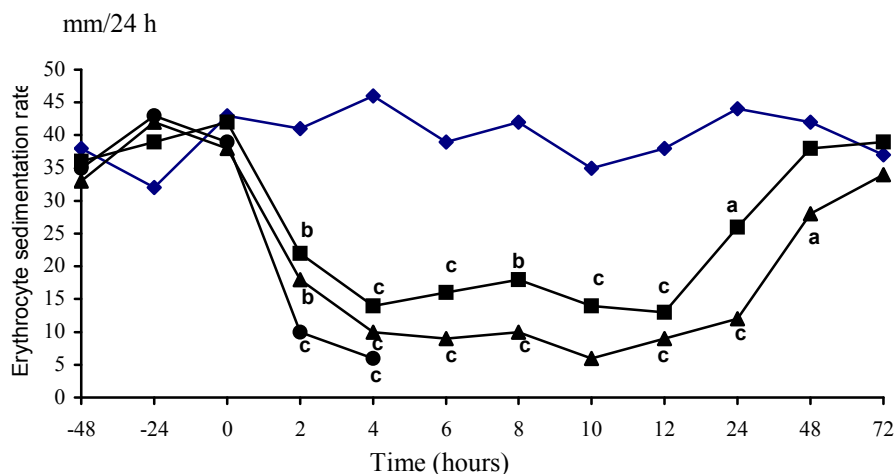


Fig. 8. Changes in erythrocyte sedimentation rate (ESR) in rabbits - untreated (controls) and treated with diniconazole at doses of 0.064 g/kg ($0.1 \times LD_{50}$, group I); 0.320 g/kg ($0.5 \times LD_{50}$, group II) and 0.639 g/kg (LD_{50} , group III). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ♦ control group; ■ group I; ▴ group II; ● group III.

2.7%, respectively, at $p < 0.001$). Lymphocyte percentage in the third group was $32.8 \pm 2.6\%$ at hour 4 ($p < 0.01$). The values returned to baseline levels at hour 48 in groups I and II. During the experiment there were no significant deviations vs. controls in the percentages of basophils (0%), eosinophils ($0.1 \pm 0.9\%$), metamyelocytes ($0.3 \pm 0.7\%$), band neutrophils ($6.3 \pm 1.7\%$) and monocytes ($3.3 \pm 1.3\%$).

The ESR (24 h) was about 37.8 ± 4.6 mm in control animals (Fig. 8). Between post treatment hours 2 and 24, ESR was lower ($22.4 \pm 2.1 - 26.6 \pm 2.8$ mm) for group I. For group II ESR values were lower in the interval 2nd - 48th hour: 18.3 ± 1.6 mm and 28.4 ± 2.6 mm respectively). At post treatment hour 4, ESR reached 6.4 ± 1.1 mm in the third group ($p < 0.001$). The slowest ESR for group I was detected at hour 12 (13.8 ± 1.1 mm; $p < 0.001$) and at hour 10 for group II (6.8 ± 0.8 mm; $p < 0.001$). There was no erythrocyte sedimentation at hour 1 and 2. A tendency towards normalization was observed between hours 48 and 72.

DISCUSSION

The experiments, performed in rabbits with increasing doses of the triazole fungicide diniconazole, showed a variable effect of the preparation upon the animal organism. The temperature curve indicates a decrease in body temperature between hours 2 and 12. It could be speculated whether this hypothermia is related to a metabolic slow-down resulting from the hypothyreosis, evidenced in cattle (Markelov and Shormanov, 1994) following Baytan (triadimenol) treatment and in humans (Machera, 1995) under the influence of flusilazole and propiconazole.

The decreased RBC counts in experimental groups, manifested between hours

4 and 48 was probably due partly to the massive haemorrhages, observed by us in hens following diniconazole administration (Binev, 2000a), in cats following treatment with Tilt (propiconazole) (Kurumbaev, 1996) and cattle (Markelov and Shormanov, 1994) under the influence of Baytan (triadimenol). The decreased RBC counts in our study could be accepted as a cause for the observed oligochromemia. This finding corresponds with the data reported by Sivertsen and Muller (1999) after treatment of mice with dialkylimidazole, 1,2-dimethyl-imidazole, 1,4-dimethylimidazole, 1,5-dimethylimidazole, 2,4-dimethylimidazole and 4,2-ethyl-4-methylimidazole as well as with the data about the effect of hexaconazole (Chen et al., 1996); cyproconazole, flusilazole, 1,2,4-triazole and propiconazole in albino mice, albino rats and humans (Machera, 1995; Celic et al., 1996; Shandrenko and Dmitrenko, 1996). It is accepted that accelerated heart and respiratory rates, observed in our experiments too, are a compensatory systemic reaction of oligochromemia (Markelov and Shormanov, 1994). The neutrophilia and lymphopenia in these trials correspond to similar changes occurring in cattle (Markelov and Shormanov, 1994), rats (Patel et al., 1998), humans (Machera, 1995). Those changes are considered by some authors to be associated with the pathomorphologically proved inflammation of the stomach and intestines resulting from the irritating effect of the peroral administration of the preparations Baytan (triadimenol) in cattle (Markelov and Shormanov, 1994), Sumi 8 2WP (diniconazole) in hens (Binev, 2000a; 2000c) and Tilt (propiconazole) in cats (Kurumbaev et al., 1996).

Our experiments showed a decreased ESR characterized with a relatively rapid

dynamics. The elucidation of the possible relationship among the ESR changes, observed by us, the decrease in globulins content and the increase in biliary salts and biliary pigments observed in diniconazole-treated hens in another study of ours (Binev, 2000b) deserves further attention. Similar facts have been reported in rats, mice and humans following treatment with cyproconazole, flusilazole, 1,2,4-triazol and propiconazole (Machra, 1995).

In conclusion, we could state that the intoxication of rabbits with the triazole fungicide diniconazole, included in the preparations Sumi 8 2VP was accompanied by clinical symptoms as hypothermia, tachycardia and polypnea. The ingestion of toxic doses (equal to one-tenth of LD₅₀, half of LD₅₀ and one LD₅₀ - oral doses for rats) resulted in haematological changes as oligochromemia, erythropenia, neutrophilia, lymphocytopenia and ESR decrease. The ingestion of a dose equal to the LD₅₀ for rats led to a lethal issue in all treated rabbits 4 hours after the treatment.

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