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Clinical toxicological investigations on acute carbofuran intoxication in quails (Coturnix coturnix)

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(Manuscript received 7 September 2016; accepted for publication 19 October 2016)

Abstract. The present study was conducted to evaluate the toxic effects of the carbamate insecticide carbofuran (Carbosan 35 ST) after experimental acute intoxication in quails (Coturnix coturnix). Experiments for monitoring of changes in clinical indices and some haematological parameters. Quails were divided into 5 groups: one control and 4 experimental. They were treated by increasing single doses of the tested pesticide: 1.05 mg/kg (experimental group I), 2.1 mg/kg (experimental group II), 5.25 mg/kg (experimental group III) and 10.5 mg/kg (experimental group IV), corresponding to 1/10 LD50, 1/5 LD50, 1/2 LD50 and LD50 oral doses for albino rats, respectively. In three consecutive days prior to the treatment (hours –48, –24 and 0) and 1, 3, 5, 7, 24 and 48 hours thereafter, the clinical status was registered to evaluate rectal body temperature, faeces excretion, locomotion, perception etn. and were obtained blood samples from v. subcutanea ulnaei or v. metatarsae ulnaei for analysis of haemoglobin content (HGB). It was found out that the tested carbamate insecticide had some toxic effects manifested clinically with hyperaemia, arexia, difficulty in focusing the eyes, salivation with thick saliva discharge, diarrhoea, generalised tremor, clonic tonic spasms (especially of cervical muscles), depression and hypochromaemia. The described changes were the most obvious thereafter, the clinical status and LD50 oral doses for albino rats, respectively.

In intoxicated quails, arexia, difficulty in focusing the eyes, salivation with thick saliva discharge, diarrhoea, generalised tremor, clonic tonic spasms (especially of cervical muscles), depression and hypochromaemia. The described changes were the most obvious thereafter, the clinical status and LD50 oral doses for albino rats, respectively. In intoxicated quails, arexia, difficulty in focusing the eyes, salivation with thick saliva discharge, diarrhoea, generalised tremor, clonic tonic spasms (especially of cervical muscles), depression and hypochromaemia. The described changes were the most obvious thereafter, the clinical status and LD50 oral doses for albino rats, respectively. In intoxicated quails, arexia, difficulty in focusing the eyes, salivation with thick saliva discharge, diarrhoea, generalised tremor, clonic tonic spasms (especially of cervical muscles), depression and hypochromaemia. The described changes were the most obvious thereafter, the clinical status and LD50 oral doses for albino rats, respectively. In intoxicated quails, arexia, difficulty in focusing the eyes, salivation with thick saliva discharge, diarrhoea, generalised tremor, clonic tonic spasms (especially of cervical muscles), depression and hypochromaemia. The described changes were the most obvious thereafter, the clinical status and LD50 oral doses for albino rats, respectively.

Keywords: quails, carbamate insecticide, carbofuran, intoxication, clinical signs

Introduction

Pesticides are a large group of chemical compounds intended to control insects (insecticides), rodents (rodenticides), weeds (herbicides), agents of fungal diseases (fungicides) etc. (Klaassen, 2008). In modern agriculture, the use of these preparations is a limiting factor for protection of plants from pests and their use serves to increase crop yields. The wide application and toxicity of these preparations is a global ecotoxicological problem (Guitart et al., 2010a). The continuous and uncontrolled pollution of soils and waters with pesticides for plant protection could have a dangerous impact by contamination of plants, some of which are consumed by humans and another part serves for feeding livestock producing main foods of animal origin. That is why the use of pesticides is a global ecotoxicological problem (Guitart et al., 2010a).

The largest pesticide group is that of insecticides, mainly represented by carbamates and organophosphate compounds. The toxicokinetics of carbamate pesticides is due to altered activity of cholinesterase, but organocarbanate compounds bind to the active site of the enzyme via reverse carbamylation (Peranantham et al., 2014).

One of the most commonly used and most toxic carbamate insecticides is carbofuran (Carbosan, Furadan, Curater). These properties of carbofuran are the cause for increased cases of intoxication in domestic animals (Martínez-Haro et al., 2008; Guitart et al., 2010a; Novotný et al., 2011), game birds, wild mammals, rodents (Ogada, 2014), fish, beneficial insects (Guitart et al., 2010b), as well as in men (Tennakoon et al., 2013; Peranantham et al., 2014) at both national and global scale. The utilisation of carbamate preparations (carbofuran) as baits for control of harmful rodents results in massive intoxications of wild birds (vultures, hawks, eagles etc.) (Mineau and Tucker, 2002a,b; Brasel et al., 2007), including quails (de Lavaur et al., 1991; Modra and Svobodova, 2009; Toll et al., 2010). Having investigated accidents with wild birds in the USA, Great Britain and Canada between 1985-1995, Mineau and Fletcher (1999) established that carbamate and organophosphate insecticides have induced the greatest number of intoxications – 520 registered cases. The main cause was negligence as treated seeds (wheat and corn) were not well buried in the ground and therefore were picked up by birds or they were secondarily intoxicated from eating earthworms contaminated with pesticides.

In previous studies of ours on blood enzyme activity in quails (Binev et al., 2014b), treated with increasing doses of the carbamate insecticide carbofuran, reduced serum cholinesterase and elevated activities of aspartate aminotransferase, alanine aminotransferase, creatine kinase and alkaline phosphatase were established. The most pronounced changes were noted in the beginning of intoxication (hours 1–3), followed by restoration of studied parameters until the 24th hour. In our country, experimental studies on the toxic effect of carbamate compounds have been carried out with chickens (Yotsev et al., 1997), but there are no data for the effects of carbamate insecticide carbofuran in wild birds, which are the commonest victims of intoxications in the nature.

The reported data and the increasing incidence of large-scale intoxications of wild birds with carbamate pesticides at national and global scale were the incentive of the present study on clinical signs and changes in some haematological parameters in quails with experimental acute intoxication with the carbamate insecticide carbofuran (Carbosan 35 ST).
Material and methods

Experimental animals. The experiments were carried out in 2016 with 30 female quails (Coturnix coturnix) with uniform gender, age 10–12 weeks and weight from 100 to 120 g. They originated and were kept in Stará Zagora City Zoo. One month before the trial, the birds were housed under uniform conditions compliant with hygienic norms. All quails were fed a ration corresponding to their species and age, and had free access to drinking water.

Tested substance. The experimental intoxication was provoked with carbofuran (Carbosan 35 ST, Agro Science – USA), containing 350 mg 2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate in 1 mL, with oral L50 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. Quails were divided into 5 groups: one control and 4 experimental (6 birds in each). They were treated on hour 0 with different single doses of the tested pesticide: 1.05 mg/kg (experimental group I), 2.1 mg/kg (experimental group II), 5.25 mg/kg (experimental group III) and 10.5 mg/kg (experimental group IV), corresponding to 1/10 L50, 1/5 L50, 1/2 L50 and L50 oral doses for albino rats, respectively.

The complete clinical status included rectal body temperature (by a digital thermometer GT 2038 Geratherm Medical, Germany), defecation, locomotion and perception (using routine clinical examination). The described clinical signs were observed from 100 to 120 g. They originated and were kept in Stará Zagora City Zoo. One month before the trial, the birds were housed under uniform conditions compliant with hygienic norms. All quails were fed a ration corresponding to their species and age, and had free access to drinking water.

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The complete clinical status included rectal body temperature (by a digital thermometer GT 2038 Geratherm Medical, Germany), defecation, locomotion and perception (using routine clinical diagnostic approaches) was monitored in all groups of quails three days before the treatment (hours –48, –24 and 0) and on post treatment hours 1, 3, 5, 7, 24 and 48. Blood was sampled from v. subcutanea ulnaris or v. metarsea for analysis of haemoglobin content on an automated analyser (Cell dyn 4500, USA).

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

The clinical examinations performed to establish the general tolerance of quails to carbofuran showed that the preparation was not toxic at a dose of 1.05 mg/kg (1/10 L50) for albino rats (experimental group I). The treated animals did not exhibit any signs of intoxication.

Observed clinical changes in birds treated at doses of 2.1 mg/kg (1/5 L50), (experimental group II), 5.25 mg/kg (1/2 L50), (experimental group III) and 10.5 mg/kg (L50), (experimental group IV) were of similar magnitude and time course. All treated birds showed arexia, difficulty in focusing the eyes, moderate salivation, diarrhoea, tremor and clonic tonic spasms (especially of cervical muscles). These signs appeared about the 1st hour after the treatment and lasted until the 3rd hour, thereafter become weaker and disappeared until the 5th hour. On the basis of data, it could be assumed that the dose of 2.1 mg/kg (1/10 L50) for albino rats was the minimum toxic dose of carbofuran for quails.

Birds which received single doses of 5.25 mg/kg (1/2 L50) (experimental group III) showed clinical signs of intoxication as early as the 15th minute and manifested depression, salivation with thick saliva discharge. Between the 20th and 40th minute, bowel incontinence occurred. Between post treatment minutes 45 and 60, nervous signs – generalised tremor, clonic tonic spasms and convulsions appeared. The described clinical signs were observed with decreasing intensity until the 5th hour after the challenge, and until the 7th hour the animals restored their physiological activity.

All animals treated at 10.5 mg/kg (L50) (experimental group IV) showed signs of intoxication after the 5th minute, similar to those observed with lower doses. All treated birds overcome the intoxication after the 7th hour.

The values of rectal body temperature (RT) (Figure 1) increased after the introduction of the preparation. The highest values in birds from groups II to IV were registered by the 1st hour 41.6±0.3 °C (p<0.01), 42.0±0.2 °C (p<0.01) and 42.3±0.3 °C (p<0.001) vs controls (40.5±0.3 °C), respectively. Except for group II, the fever was preserved by the 4th hour as well. By the 5th hour, the clinical parameter has returned to its normal values.

The amount of haemoglobin (Hgb) prior to and after treatment of quails is presented on Figure 2. After intoxication with carbofuran Hgb decreased, and this was expressed between the 5th and 7th hours in group II, most obviously by the 5th hour 165±14.3 gl
alternating discharge, tremor (Goad et al., 2004). Returned to its normal values. The fever was probably associated to hour and within hours 45. Exposed established in previous studies of ours 10.5 mg/kg, quails we assumed that the tolerable dose for this species was 1.05 mg/kg. The treated birds did not exhibit clinical signs of intoxication, hence The studied intoxication were comparable to those reported by Brasel (2007), Modra and Svobodova (2009) and Tall et al. (2010) in spontaneous intoxication of wild birds with carbamate insecticides. The study carried out to establish the total tolerance to carbofuran showed that the preparation was not toxic for quails at a dose of 1.05 mg/kg (1/10 LD₅₀ for albino rats) (experimental group I). The treated birds did not exhibit clinical signs of intoxication, hence we assumed that the tolerable dose for this species was 1.05 mg/kg.

According to our study, clinical signs of intoxications appeared between the 1st and the 3rd hour in birds from experimental group II, treated with 2.1 mg/kg carbofuran, i.e. 1/5 LD₅₀. These data allowed assuming that this was the minimum toxic dose of the preparation for quails.

With regard to the species-specific susceptibility to the tested carbamate insecticide, the tolerable and minimum toxic dose for dogs treated with similar doses of carbofuran (Binev et al., 2014a), were twice lower.

Our experiments showed also that the minimum lethal dose was 10.5 mg/kg, equal to LD₅₀ for albino rats as well as that it was twice higher than that for dogs (5.25 mg/kg ½ LD₅₀ for albino rats) established in previous studies of ours (Binev et al., 2014a). All dogs exposed to 10.5 mg/kg (LD₅₀ for albino rats) died between the 30th and 45th minute (Binev et al., 2014a). Clinical intoxication with carbofuran in quails from experimental group IV (treated with 10.5 mg/kg) occurred about post treatment min 15-30, with most obvious signs by post treatment hour 1.

The body temperature curve in experimental group I showed that low doses of carbofuran (1/10 LD₅₀) did not influence it. As doses increased, it also went higher with peak values detected by the 1st hour and within hours 35, then the studied clinical parameter returned to its normal values. The fever was probably associated to enhanced general metabolism by the exposure to carbamate pesticides (Kim et al., 2004). From the other hand, thermoregulation could be impaired by the neurotoxic effects of these compounds (Goed et al., 2004).

Changes in the behaviour and general condition observed by us and others (Tall et al., 2010): arexia, anxiety, diarrhoea, polydipsia, tremor, clonic tonic spasms, depression, salivation with thick saliva discharge, miosis, impaired locomotor coordination, inability to stand, failling, incontinence, generalised tremor, convulsions, movement within a circle, paresis and paralysis of extremities alternating with depression and somnolence, turning of the head, spasms, delayed reflexes and sensitivity, are due to irritation of cholinergic receptors by increased acetylcholine which caused the observed neurotoxic symptoms (Modra and Svobodova, 2009).

In the course of acute carbofuran intoxication, the amount of haemoglobin in treated groups of quails was also altered. After the treatment, haemoglobin content decreased proportionally to the increase of the dose. Maximum hypochromaemia was present one hour after the exposure.

Hypochromaemia in experimental groups were due, from one hand, to multiple haemorrhages on parenchymal organs established in gross anatomy and other studies (Rizos et al., 2004; Guitart et al., 2010a). On the other hand, the reduced haemoglobin was associated to lower red blood cell counts due to depletion of liver glutathione (GSH) which prevents their haemolysis (Adhikari et al., 2004). A third possible mechanism of hypochromaemia is hypoxaemia-stimulated vasconstriction with release of catecholamines, leading to redistribution in blood composition manifested by leukocytosis and erythroplaenia. The intoxication with carbamate preparations and catecholamine increase is additionally due to increased cholinergic stimulation of receptors consequently to inhibited cholinesterase activity (Peranantham et al., 2014). Previous data of ours in dogs (Binev et al., 2014a) and data from other authors (Adhikari et al., 2004) reporting reduction of red blood cell counts could be assumed as a cause of observed hypochromaemia.

The results of our studies on the effects of acute carbofuran intoxication in quails differ from those of Zhelev (2004) reported in poultry and pigs. The author reported hyperhaeromaemia and erythrocritosis, correlating to increased haematocrit, although according to other authors (Zaahkouk et al., 2000) blood haemacrit was decreased consistently with hypochromaemia and erythroplaenia.

Conclusion

The study performed to evaluate the toxic effects of increasing doses of the carbamate pesticide carbofuran in quails (1/20 LD₅₀, 1/10 LD₅₀, 1/5 LD₅₀, 1/3 LD₅₀ and 1/2 LD₅₀) revealed that clinical signs of intoxication could be summarised as fever, arexia, difficulty in focusing the eyes, salivation with thick saliva discharge, diarrhoea, generalised tremor, clonic tonic spasms (especially of cervical muscles), and depression. The established hypochromaemia was also added to observed signs. The described changes were the most obvious in the beginning of intoxication (hours 1 and 3), and consequently the studied parameters regained their control values. The tolerated dose of carbofuran established in this study was 1.05 mg/kg (1/10 LD₅₀ for albino rats), the minimum toxic dose was 2.1 mg/kg (1/5 LD₅₀ for albino rats), and the minimum lethal dose - 10.5 mg/kg, equal to LD₅₀ for albino rats.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This study was funded by project №19/2006 and was supported.
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