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Research Article

Morphological Investigations of Experimental Acute Intoxication with the Anticoagulant Rodenticide Bromadiolone in Pheasants

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ABSTRACT

Morphological investigations were performed to observe the changes after experimental acute intoxication with the anticoagulant rodenticide bromadiolone in pheasants. The study was performed with 8 groups of pheasants treated with increasing doses of the tested preparation: 5 mg/kg (group I), 10 mg/kg (group II), 20 mg/kg (group III), 30 mg/kg (group IV), 40 mg/kg (group V), 50 mg/kg (group VI), 60 mg/kg (group VII) and 70 mg/kg (group VIII). All birds from groups I to V have survived the intoxication whereas those from groups VI, VII and VIII have died. During the intoxication, inappetence, accelerated and difficult breathing, adynamia, watery blood discharge from the beak were observed. All pheasants with fatal outcome and the survivors, which were euthanized after the experiment (day 20) were necropsied and gross changes in the liver, the lungs and kidneys were described. Liver alterations varied from strong hyperaemia and activation of the monocytic-macrophageal system to diffuse vacuolar or granular parenchymal dystrophy, as well as necrobiotic to necrotic changes, intra- and inter-lobular haemorrhages, perivascular mononuclear proliferations and bile duct hyperplasia. Lungs exhibited congestive hyperaemia, oedema in the interstitium and the mucous coats of bronchi and parabronchi, desquamation of epithelial cells in bronchioles and lung parenchymal haemorrhages. In the kidney parenchyma, congestive hyperaemia and haemorrhages were seen, varying within a broad range from karyolysis and karyopyknosis in epithelial tubular cells to cellular desquamation and disintegration and necrosis. All observed changes in parenchymal organs were dose-related, being more pronounced in pheasants treated with higher doses of the tested rodenticide.

Key Words: Anticoagulant rodenticide, bromadiolone, pheasants, acute intoxication, morphological investigations

ÖZET

SÜLÜNLERDE ANTİKOAGÜLAN RODENTİSİT BROMADİOLONE İLE OLUŞTURULAN DENEYSEL AKUT İNTOKSİKASYONUN MORFOLOJİK İNCELENMESİ

Sülünlerde antikoagülan rodentisit bromadiolone kullanılarak geliştirilen deneysel akut intoksikasyondan sonra değişimleri gözlemlemek amacıyla morfolojik incelemeler yapılmıştır. Çalışmada 8 grup sülün artan dozlarda incelemeye alındı: 5 mg/kg (Grup 1), 10 mg/kg (Grup 2), 20 mg/kg (Grup 3), 30 mg/kg (Grup 4), 40 mg/kg (Grup 5), 50 mg/kg (Grup 6), 60 mg/kg (Grup 7) ve 70 mg/kg (Grup 8). Birinci ve 5. gruplar arasındaki kuş gruplarının hepsi

intoksikasyondan hayatta kalırken; 6., 7. ve 8. grupların öldüğü saptanmıştır. İntoksikasyon sırasında, iştahsızlık, hızlı solunum ve solunum güçlüğü, bitkinlik, gagadan sulu kan geldiği gözlenmiştir. Deney sonucunda (20 gün) ötenazi yapılarak nekropsisi yapılan hayatta kalan sülünlerin ve ölen sülünlerin nekropsilerinde dalak, akciğer ve böbreklerde değişiklikler saptandı. Dalakta şiddetli hiperemi ve monositik-makrofaj sisteminin aktivasyonu sonucu diffuz vakuoler ya da granüler parenkimal distrofi, intra ve inter lobuler hemorajiler, perivasküler mononükleer proliferasyonlar ve safra kanalı hiperplazisi görülmüştür. Akciğerlerde konjestif hiperemi, interstitiyum, bronş ve parabronşların mukoz membranlarında ödem, bronşiollerde bulunan epitel hücrelerde deskuamasyon ve parenkimal hemorajiler görülmüştür. Böbrek parenkiminde, konjestif hiperemi ve hemorajiler görülmüş, epitelyal tubuler hücrelerinde değişen derecelerde karyolizis ve karyopiknozis alanları, hücresel deskuamasyon ve parçalanma ve nekroz odakları görülmüştür. Parenkimal organlarda gözlenen tüm değişiklikler doza bağlı olarak değişmekle birlikte, rodentisitin yüksek dozda uygulandığı sülünlerde değişimler daha belirgin olarak seyretmiştir.

Anahtar Kelimeler: Antikoagülan rodentisit, bromadiolone, sülün, akut intoksikasyon, morfolojik incelemeler

Introduction

Anticoagulant rodenticides are the most commonly used pesticides to control harmful rodent populations (Albert et al., 2009). Other compounds used with the same purpose, are the derivatives of bromethalin, sodium fluoroacetate and cholecalciferol (Dorman et al., 1990; Fry and Forman, 2004; Sherley, 2004). They are less frequently used, while rodenticides containing zinc and arsenic are prohibited. The mechanism of anticoagulant rodenticides' toxic effect consists in inhibition of vitamin K_1 reductase in liver microsomes, thus interrupting the cell turnover of vitamin K_1. Consequently, the liver stores of active vitamin K_1 are depleted and the synthesis of coagulation factors (II, VII, IX and X), involved in extrinsic and intrinsic coagulation cascade pathways stops (Mount, 1988). Both domestic and wild animals are intoxicated by two principal routes: ingestion of anticoagulant-containing baits by accident (first option) or after eating dead rodents (second option) (Nikolov and Binev, 2008; Stone et al., 1999; Valchev, et al., 2008). The lack of side effects as unpleasant odour and taste is another precondition for intoxications in humans and animals (Todorov, 2006). Mass intoxications in birds (seagulls, geese, sparrows, quails, ducks, pheasants, kiwi, owls, falcons, magpies, parrots, pigeons etc.) after placing baits against mice (*Mus domesticus*), European water voles (*Arvicola terrestris*) and rats (*Rattus norvegicus, R. rattus*) are reported by Eason et al. (2002), Godfrey (1985), Spurr (1993) and Stone et al. (1999).

Previous studies of ours on spontaneous (Binev et al., 2005) and experimental intoxications (Valchev et al., 2009a; 2009b) with bromadiolone in dogs described the clinical and haematological alterations in this animal species. It was shown that the intoxication was accompanied by hypothermia, tachycardia, polypnea, erythropaenia, reduced haematocrit, prolonged activated partial thromboplastin and prothrombin times, increased activities of aminotransferases (AST and ALT), blood glucose, total bilirubin, urea and creatinine. In wild birds (falcons, owls, eagles, hawks) intoxicated with warfarin, brodifacoum and diphacinone, the following gross changes were observed: subcutaneous haemorrhages, pulmonary, pericardial, intra- and intermuscular haemorrhages, alimentary tract haemorrhages and pale mucous coats (Stone et al., 1999). Diffuse bleeding and non coagulated blood in thoracic and abdominal cavities were reported in chickens poisoned with coumafuryl (Munger et al., 1993). Pathomorphological changes in the liver were performed after coumarin challenge in rats and mice (Evans et al., 1989; Jack et al., 1996; Lake and Evans, 1993; Lake and Grasso, 1996) and diphacinone intoxication in lambs (Del Piero and Poppenga, 2006) and dogs (DuVall et al., 1989). In these studies, depending on the amount of ingested toxic compound, liver parenchyma changes were of various extents.

A number of kidney injuries are reported in human patients submitted to anticoagulant therapy with coumarin and its derivatives

(warfarin and fluindione) – interstitial nephrites, renal failure, glomerulonephritis (Beauchamp et al., 2008; Brodsky et al., 2009; Kapoor and Bekali-Saab, 2008; Remková et al., 2010). In dogs intoxicated with coumarin rodenticides, histopathological changes consisted in dystrophic damage of kidney parenchyma (Todorov, 2006), and hyperaemia, oedema and haemorrhages in lungs (DuVall et al., 1989; Mount, 1988; Palmer et al., 1999).

Depending on their chemical structure, rodenticides are divided into 2 principal groups [hydroxycoumarine and indandione (chlorophacinone, diphacinone, pindone and valone)]. From their part, hydroxycoumarin rodenticides are subdivided into first generation (coumachlor, coumafuryl, coumatetralyl and warfarin) and second generation (brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen) compounds (Valchev et al., 2008).

Having in mind the importance of the occurrence of spontaneous intoxications in pheasants after ingestion of baits for rodents containing anticoagulant rodenticides and the lack of information about morphological changes after experimental intoxication of dicoumarin rodenticide bromadiolone in pheasants, the present study aimed to establish the morphological changes induced by the substance in this avian species.

Materials and Methods

Experiments were performed with 120 common pheasants from both genders, at the age of 1-1.5, weighing 0.950-1.5 kg. The birds were divided into 8 experimental groups (n=15), treated on day with increasing single doses of bromadiolone, as follows: 5 mg/kg (group I), 10 mg/kg (group II), 20 mg/kg (group III), 30 mg/kg (group IV), 40 mg/kg (group V), 50 mg/kg (group VI), 60 mg/kg (group VII) and 70 mg/kg (group VIII). The preparation was administered intraingluvially by an oesophageal probe. The doses were chosen on the basis of LD_{50} of brodifacoum in pheasants – 10 mg/kg (Goldfrey, 1985) as data about median lethal dose of bromadiolone in pheasants is not available. One month prior to the experiment, the birds were placed in separate aviaries by groups in one common premise, and were fed and reared uniformly with free access to drinking water. All pheasants were treated against parasites with Piperatrin (*piperazine hexahydrate* – 20 g/100 ml, VetProm, Radomir, Bulgaria). Before and throughout the experiment, pheasants were fed wheat, corn and sunflower seeds.

All pheasants with lethal issue and those that survived the intoxication, were necropsied by the end of the study (day 20) after euthanasia by cervical dislocation. Specimens were collected from the liver, kidneys and lungs for histological examination. (The samples were routinely processed, cut at about 5-μm-thickness (Leica RM 2235) and then stained with haematoxylin-eosin).

Results

Pheasants treated with 5 mg/kg, 10 mg/kg, 20 mg/kg and 30 mg/kg doses of bromadiolone did not exhibit any changes in their physical health status.

During the course of intoxication, groups that received 40 and 50 mg/kg broma-diolone showed in appetence, ruffled feathers, accelerated and difficulty breathing with the beak open. The changes were observed between 7th and 13th days. Within the 10th and 13th day, two pheasants treated with 40 mg/kg have died, and three fatal outcomes were observed in the group treated with 50 mg/kg. Birds treated with 60 mg/kg bromadiolone, apart aforementioned clinical signs, showed also conjunctiva bleeding and died on the 13th day of intoxication. Pheasant that received 70 mg/kg bromadiolone, exhibited wing drooping, adynamia, watery noncoagulated blood discharge from the beak (Figure 1). The lethal issue in this group occurred on the 11th day of treatment.

Gross changes

The necropsy of pheasants treated with 5, 10 and 20 mg/kg bromadiolone (groups I, II and III) demonstrated hyperaemia of serous coats of viscera. The vent feathers of birds from group III were stained with faeces. The duodenum was filled with grey-yellowish content and gas

bubbles (catarrhal inflammation). Pheasants treated with 30 mg/kg (group IV) showed massive haemorrhages on pectoral muscles on breast bone tip and inner side (Figure 2). At the boundary between gizzard and proventriculus, the mucosa had a red discoloration due to imbibition and haemorrhages. Bone marrow was pale rose in colour. Pheasants treated with 40 mg/kg bromadiolone (group V) had massive subcutaneous haemorrhages in the region of the neck, anteriorly to the crop and in thigh muscles region. The changes at the boundary between the gizzard and proventriculus and in bone marrow colour were similar to those in group IV. The lungs in pheasants treated with 30 and 40 mg/kg bromadiolone (groups IV and V) were hyperaemic. A foamy discharge was observed from the cut surface of lungs (oedema). Pheasants treated with 50 mg/kg bromadiolone (group VI), showed haemorrhages on pectoral and thigh muscles. Coronary blood vessels were overfilled with blood, and cardiac musculature had a marbled appearance and was mottled with pale spots. The duodenal mucosa was strongly reddened and mottled with petechiae and striate haemorrhages. Haemorrhages were observed in the other parts of intestines. Visceral serous coats and air sacs were purple in colour. The lungs were hyperaemic, with single petechiae, and the trachea was filled with foamy reddish fluid. Pheasants treated with 60 mg/kg bromadiolone (group VII) had markedly extensive haemorrhages on pectoral and thigh muscles than those in birds from other groups. The pericardium was mottled with petechiae. In intestines, both petechiae and striate haemorrhages were observed. The mucosa of the proventriculus was purple, scattered with haemorrhages, and the cuticle of the gizzard was strongly keratinized, with necrotic areas at some places. The bone marrow was with pulpy creamy consistence. A large amount of noncoagulated blood was observed in the pleuroperitoneal cavity. The intestinal mucosa was mottled with petechiae. Pheasants treated with 70 mg/kg bromadiolone (group VIII) showed a jelly-like reddish-purple swellings. The serous coats of viscera and air sacs were also purple-coloured. The pericardium was filled with dark purple opaque fluid and the epicardium was spatter with petechiae. The alterations in pleuroperitoneal cavity, the intestines and bone marrow structure were similar to those described in group VII. Lungs of pheasants treated with 60 and 70 mg/kg bromadiolone were with dark red spots, and after cutting, a foamy blood discharge was observed.

Macroscopic changes in liver colour in experimental groups varied within a broad range depending on the dose of rodenticide. Birds that received 5, 10, and 20 mg/kg bromadiolone (groups I, II and III) had dark red livers, whereas in those from groups IV to VI, it was marbled, brown-yellowish in colour, with frail consistency and mottled with petechiae and striate haemorrhages. In birds from groups VII and VIII, the liver's colour was ochre and scattered with pale spots (fatty dystrophy and necrosis). The gallbladder was enlarged due to overfilling with a large amount of bile whose colour varied from dark green (groups I, II and III) to dark purple (groups IV to VIII).

The gross investigation of lungs revealed various and dose-dependent changes similar to those in liver. Pheasant from groups I, II and III had hyperaemic lungs, while those from groups IV, V and VI – rose-red in colour, mottled with petechiae and with foamy fluid discharge from the cut surface (pulmonary oedema). Lungs of pheasant treated with 60 and 70 mg/kg bromadiolone were with round corners, dark red colour, scattered with haemorrhages and filled with serous bloody exudate with a large amount of non-coagulated blood around.

Gross anatomy observations of kidneys from pheasants treated at 5, 10 and 20 mg/kg bromadiolone showed the presence of urates. In birds treated with 30, 40 and 50 mg/kg bromadiolone, the kidneys were hyperaemic, with frail consistency and petechiae. The kidneys of pheasants from groups VII and VIII were enlarged, of red purple colour and increased fragility.

Figure 1. Watery noncoagulated blood from the nostrils of a pheasant, treated with 70 mg/kg bromadiolone.
Şekil 1. 70 mg/kg bromadiolone uygulanan sülünün burun deliklerinden gelen sulu ve pıhtılaşmamış kan.

Figure 2. Haemorrhages on the breast bone top in a pheasant treated with 30 mg/kg bromadiolone.
Şekil 2. 30 mg/kg bromadiolone uygulanan sülünün göğüs kemiğinde hemorajiler.

Histopathological changes

Microscopic examination of liver of pheasant treated with 5 mg/kg bromadiolone demonstrated a marked enlargement and overfilling with blood of capillaries, central and interlobular veins, as well as atrophy of the parenchyma on pressure. Perivascular spaces of Disse were also very enlarged and filled with blood. An initial form of vacuolar dystrophy was visualized. In pheasants treated with 10 mg/kg, changes were necrobiotic at single areas, situated in small groups of hepatocytes. Karyolysis and vacuolar dystrophy of the cytoplasm of hepatocytes was also found out. In the birds from group III apart the generalized hyperaemia and strongly activated capillary endothelium, intra- and inter- lobular haemorrhages were observed. Interlobular connective tissue was markedly swollen. Liver cells were associated and their trabecular structure was disarranged. Necrobiotic events in the hepatocytes from group IV were more pronounced; micronecroses were also present. Pheasants from group V exhibited all aforementioned alterations and also perivascular mononuclear proliferations. On the background of all these changes of liver structure, pheasant treated with a dose of 50 mg/kg showed a generalized hyperplasia of bile ducts (Figure 3). A strongly impaired liver structure was detected in birds from groups VII and VIII. In them, multiple necrobiotic to necrotic foci were found out (Figure 4).

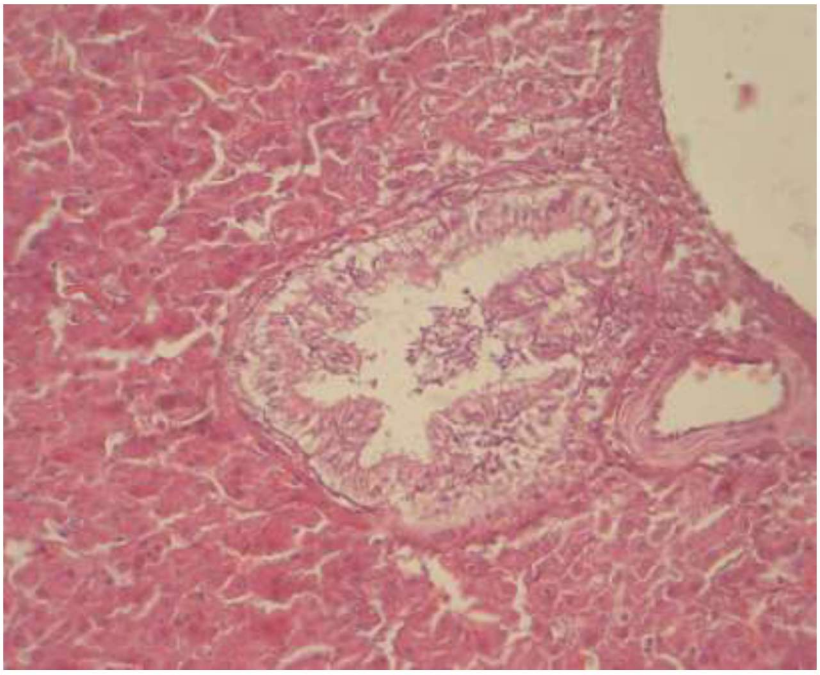

Figure 3. Liver of a pheasant, treated with 50 mg/kg bromadiolone. Hyperplasia of bile ducts.
Şekil 3. 50 mg/kg bromadiolone uygulanan sülünde dalak. Safra kanallarında hiperplazi.

The observed histopathological changes in lungs in birds treated with 5, 10 and 20 mg/kg bromadiolone consisted in strong congestive hyperaemia, interstitial oedema, swollen mucous coats of bronchi and parabronchi, and a weak desquamation of epithelial cells in bronchioles. Birds treated with higher doses bromadiolone (30, 40 and 50 mg/kg) exhibited also small haemorrhages in the interstitium and presence of siderocytes (Figure 5). In groups

treated with maximum bromadiolone doses (60 and 70 mg/kg), extensive haemorrhages of the lung parenchyma were found out (Figure 6).

Histological examination of the kidney parenchyma in groups I, II and III showed a strong generalized hyperaemia and small interstitial haemorrhages. The capillary endothelium was activated. The cells of proximal tubules were imbibed, disintegrated and at some foci, desquamated by basal membranes. Increased doses of the tested anticoagulant rodenticide (30, 40 and 50 mg/kg) resulted in strong dystrophy of epithelial cells, vacuolization of the cytoplasm, karyolysis and karyopyknosis of nuclei of tubular epithelial cells (Figure 7). Pheasants from groups VII and VIII, treated at the highest doses, exhibited necrobiotic and necrotic changes (Figure 8).

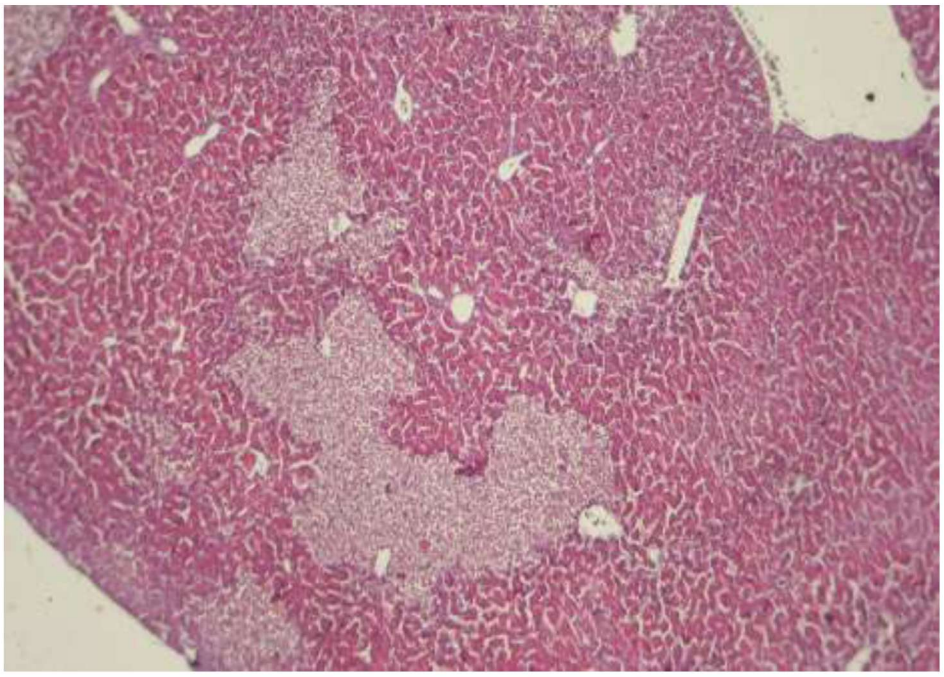

Figure 4. Liver of a pheasant, treated with 60 and 70 mg/kg bromadiolone. Multiple necrotic areas.
Şekil 4. 60 ve 70 mg/kg bromadiolone uygulanan sülünde dalak. Çoklu nekrotik alanlar.

Discussion

The established accelerated and difficulty breathing in all intoxicated birds, independently of the administered dose of the anticoagulant rodenticide could be assumed to be a compensatory mechanism of the effect of multiple massive haemorrhages, occurring secondary to the impaired blood coagulation mechanism (Del Piero and Poppenga, 2006; Kohn et al., 2003; Mount et al., 1988; Petterino and Paolo, 2001). As a result, substantial changes in haematological parameters (oligochromaemia, erythropaenia, reduced haematocrit) and prolonged prothrombin time occurred, as reported in other studies on bromadiolone intoxications of ours (Binev et al., 2005; Valchev et al., 2009a; 2009b) as well after poisoning with brodifacoum and diphacinone (Kohn et al., 2003; Petrus and Henik, 1999; Sheafor and Couto, 1999) in dogs and cats and with brodifacoum in hawks, wild ducks, eagles and crows (Bailey et al., 2005; Howald, 1997; James et al., 1998; Massey et al., 1997; Murray and Tseng, 2008). The occurring haematological changes are responsible for reduced blood oxygenation which is compensated by acceleration of breathing rate. On the other hand, the observed

morphological changes of lung parenchyma in pheasants (congestive hyperaemia, desquamation of epithelial cells of bronchioles and haemorrhages in lung parenchyma) in dogs (Valchev et al., 2009a; 2009b), lambs and birds (Del Piero and Poppenga, 2006; DuVall et al., 1989; Palmer et al., 1999; Rattner et al., 2011; Todorov, 2006), the congestion, hyperaemia, oedema, alveolar macrophages migration, haemorrhages aggravate further the respiration that apart being accelerated, becomes also difficult.

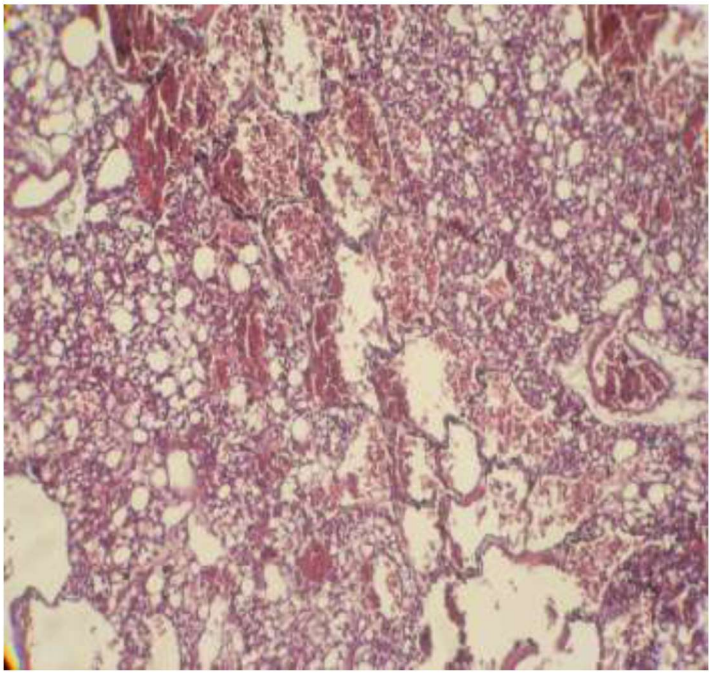

Figure 5. Lung of a pheasant, treated with bromadiolone at 30, 40 and 50 mg/kg m. Strong hyperaemia and haemorrhages and siderocytes in the lung parenchyma.

Şekil 5. 30, 40 ve 50 mg/kg bromadiolone uygulanan sülünde akciğer. Akciğer parenkiminde şiddetli hiperemi ve hemoraji ile siderositler.

Our observations on changes of liver parenchyma after bromadiolone intoxication – marked dilatation and filling of capillaries, central and interlobular veins with blood, atrophy, vacuolar dystrophy, necrobiosis, intra- and interlobular haemorrhages, perivascular mononuclear proliferations and bile duct hyperplasia – are similar to data reported by other researchers for hepatocellular necrosis (Del Piero and Poppenga, 2006; Petterino and Paolo, 2001), of hepatocytes' vacuolation (DuVall et al., 1989), necrosis, fibrosis and hydropic alterations in hepatocytes (Karanth and Nayyar, 2003). Various extent of dystrophy were reported in the liver of rats and mice after challenge with coumarin – vacuolar degeneration of hepatocytes, bile duct hyperplasia, necrotic hepatocellular changes, impaired trabecular structure (Jack et al., 1996; Lake and Evans, 1993; Lake and Grasso, 1996).

Observed morphological changes are due, from one part, to the direct toxic effect of 4-hydroxycoumarin derivatives on liver. The second generation anticoagulant rodenticides are characterized by enhanced accumulation and prolonged persistence in the liver compared to first generation compounds (Huckle et al., 1988). On the other hand, dystrophic changes could be attributed to anaemia and hypoxia, resulting from the developing bleeding diathesis in this intoxication (Del Piero and Poppenga, 2006).

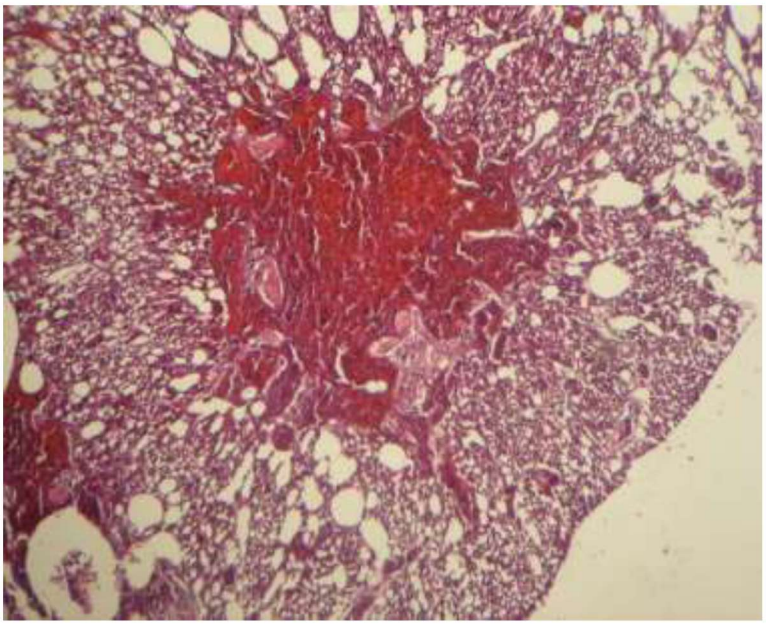

Figure 6. Lung of a pheasant, treated with bromadiolone at 60 and 70 mg/kg m. Strong hyperaemia and haemorrhages in the lung parenchyma.

Şekil 6. 60 ve 70 mg/kg bromadiolone uygulanan sülünde akciğer. Akciğer parenkiminde şiddetli hiperemi ve hemorajiler.

The morphological changes in lungs (congestive hyperaemia, oedema in the interstitium and in mucous coats of bronchi and parabronchi, desquamation of the epithelium of bronchioles, haemorrhages in lung parenchyma) are similar to data reported din dogs (DuVal et al., 1989; Palmer et al., 1999; Todorov, 2006), lambs (Del Piero and Poppenga, 2006), birds (Rattner et al., 2011) and humans (Olmos and López, 2007). The observed changes were dose-dependent and secondary to rodenticide-impaired haemocoagulation (Mount, 1988). The clinical expression of impaired morphology of lungs in pheasants is the accelerated difficult breathing, and watery noncoagulated bloody discharge from the beak.

The kidney parenchymal changes observed by us (hyperaemia, haemorrhages, activation of the capillary endothelium, imbibition, disintegration and partial desquamation, vacuolar dystrophy, karyopyknosis, karyolysis, necrosis of epithelial cells of proximal renal tubules) provided evidence for the substantial, dose-dependent toxic effect of bromadiolone on renal parenchyma. Our data correspond to findings in other animal species: dogs (DuVall et al., 1989; Todorov, 2006), lambs (Del Piero and Poppenga, 2006), birds (Rattner et al., 2011) and in humans (Olmos and López, 2007). Similar renal changes (vacuolar and granular degeneration) have been reported after coumarin poisoning (Jahad and Saddiiq, 2010). These damages could be explained by the nephrotoxic effect of anticoagulant rodenticide on renal tubules during the excretion of the compound, as also observed in intoxications with bromadiolone (Jeanted et al., 1991; Revathi and Yogananda, 2006), brodifacoum (Bachmann and Sullivan, 1983) and floucumafen (Huckle et al., 1989). Renal ischaemia, secondary to the numerous haemorrhages could be outline as an additional cause for observed morphological changes (DuVall et al., 1989).

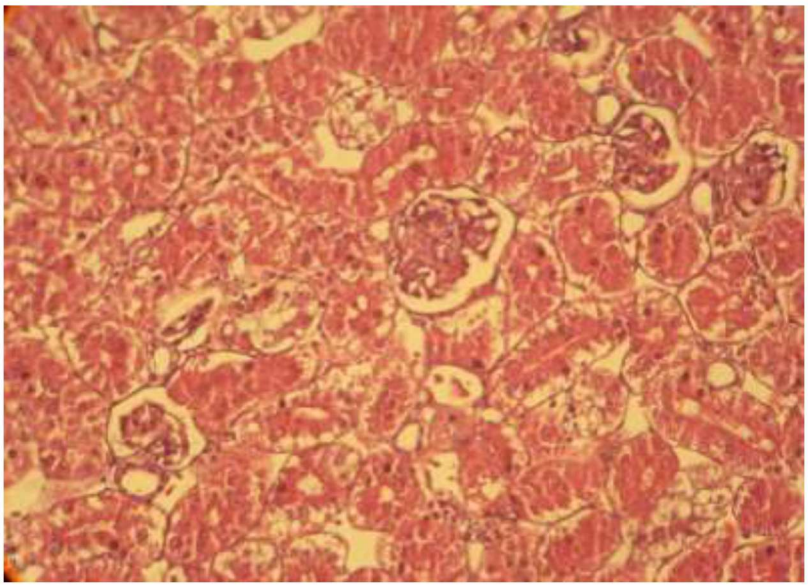

Figure 7. Kidney of a pheasant, treated with bromadiolone at 30, 40 and 50 mg/kg. Vacuolization of the cytoplasm with karyolysis and karyopyknosis of tubular epithelial cells.
Şekil 7. 30, 40 ve 50 mg/kg bromadiolone uygulanan sülünde böbrek. Sitoplazmada vakuolizasyon ile tubuler epitelyal hücrelerde karyolizis ve karyopiknozis.

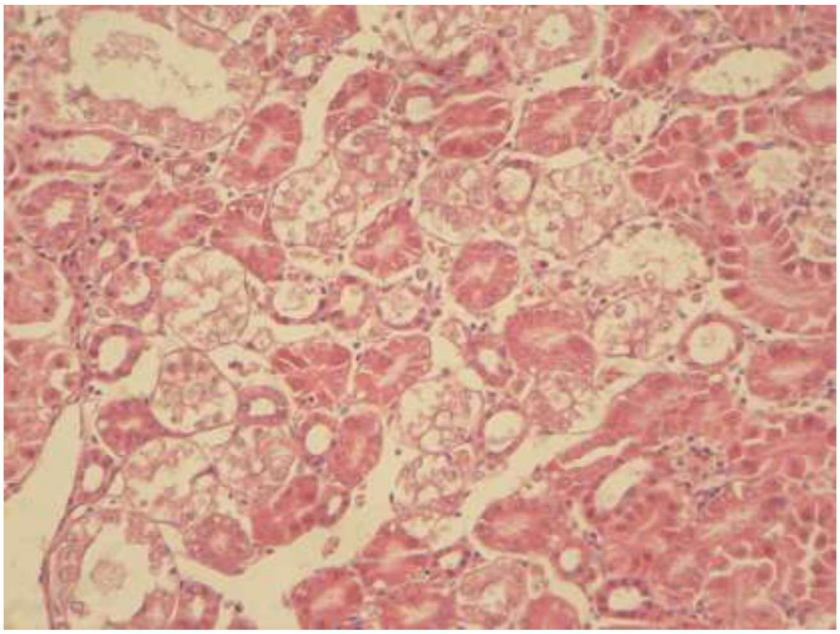

Figure 8. Kidney of a pheasant, treated with bromadiolone at 60 and 70 mg/kg. Necrobiotic and necrotic changes in tubular epithelial cells
Şekil 8. 60 ve 70 mg/kg bromadiolone uygulanan sülünde böbrek. Tubuler epitelyal hücrelerde nekrobiyotik ve nekrotik değişimler.

Conclusion

1. The experimental acute intoxication of pheasants with increasing doses of the anticoagulant rodenticide bromadiolone (5, 10, 20, 30, 40, 50, 60 and 70 mg/kg м) resulted in considerable gross and histopathological changes. They were observed in all treated birds regardless of the used dose, but the extent of damage was dependent on the dose.

2. The liver was brown-yellowish, with fragile consistency, multiple haemorrhages, fatty dystrophy and much enlarged gallbladder. Histopathological findings consisted in strong dilatation and blood-filled capillaries, central and interlobular veins, intra- and inter-lobular haemorrhages, perivascular mononuclear proliferations and bile duct hyperplasia.

3. The toxic effect of bromadiolone was manifested by substantial macroscopic (hyperaemia, massive haemorrhages, pulmonary oedema, serous bloody exudate and a large amount of noncoagulated blood) and histological changes (congestive hyperaemia, oedema in the interstitium and the mucous coats of bronchi and parabronchi, desquamation of epithelial cells in bronchioles, lung parenchymal haemorrhages) in the lungs of treated pheasants.

4. Gross anatomy findings in kidneys consisted in urate calculi, hyperaemia, petechiae, whereas microscopic changes of renal parenchyma included hyperaemia, haemorrhages, activation of the capillary endothelium, imbibition, disintegration, desquamation, vacuolar dystrophy, karyopyknosis, karyolysis, necrosis of epithelial cells or proximal renal tubules.

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