CASE REPORT

An incidence of acute nitrate-nitrite poisoning in a Guinea pig (*Cavia porcellus*) colony

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Özet

Rajesh NV, Kumar V, Ganapathi P, Thangapandiyan M. Bir kobay (*Cavia porcellus*) kolonisinde akut nitrat-nitrit zehirlenmesi. **Eurasian J Vet Sci, 2013, 29, 2, 106-109**

Bir kobay (*Cavia porcellus*) kolonisinde akut nitrat-nitrit zehirlenmesine bağlı olarak 88 adetinden 28 adetinin spontan olarak öldüğü vaka sunuldu. *Cichorium intybus* bitkisinin yeşil yapraklarının yenmesine bağlı olarak nitrat-nitrit zehirlenmesinin neden olduğunu ölümler düşünüldü. 28 adet kobaydaki belirgin belirtiler ise doğal deliklerden akan çikolata renkli kan, siyanotik mukoz membranlar, midriyazis ve şişmiş karın olarak gözlendi. 28 hayvanın nekropsisinde büyük viseral organlarda kanamalar, böbrekte tubuler nefroz, dalakta lenfoid yapı, karaciğerde hidrofobik değişiklikler ile nekroz, barsaklarda hemaraji ve akciğerde histopatalojik değişiklikler. Nitrat-nitrit zehirlenmesi spektrofotometrik olarak doğrulandı. *C. intybus* toksikolojik analizinde %2.1 g nitrat, %1.0 g nitrit ve 80 ppb sitrinin varlığı belirlendi. Barsak boşluğu 10 ppm nitrat ve 1 ppm nitrit içermekte olduğu belirlendi. Bilindiği kadarı ile mevcut vaka, *C. intybus* yeşil yapraklarının yenmesine bağlı kobaylarda akut nitrat-nitrit zehirlenmesinin bildirildiği ilk vakadır.

Anahtar kelimeler: Nitrat-nitrit zehirlenmesi, *Cichorium intybus*, kobay

Abstract

Rajesh NV, Kumar V, Ganapathi P, Thangapandiyan M. An incidence of acute nitrate-nitrite poisoning in a Guinea pig (*Cavia porcellus*) colony. **Eurasian J Vet Sci, 2013, 29, 2, 106-109**

We present an incidence of acute nitrate-nitrite poisoning in a guinea pig (*Cavia porcellus*) colony which resulted in the substantial and spontaneous death of 28 out of 88 guinea pigs. The cause of death was traced to nitrate-nitrite toxicity from ingestion of the green leaves of overfertilized *Cichorium intybus*. The predominant symptoms in the 28 guinea pigs were chocolate brown bloody discharge from all natural orifices, cyanotic mucous membrane, mydriasis, and bloated abdomen. Necropsy of all the 28 animals showed hemorrhage in all visceral organs grossly, and tubular nephrosis of the kidney, lymphoid depletion in the spleen, hydropic changes and necrosis in hepatocytes, and hemorrhages in the intestine and lungs histopathologically. Nitrate-nitrite toxicity was confirmed spectrophotometrically. The toxicological analysis of the feed samples of *C. intybus* revealed 2.1 g% nitrate, 1.0 g% nitrite, and 80 ppb citrinin. The intestinal loop had 10 ppm of nitrate and 1 ppm of nitrite. To the best of our knowledge, this is the first report of acute nitrate-nitrite poisoning in guinea pigs owing to the ingestion of the green leaves of overfertilized *C. intybus*.

Keywords: Nitrate-nitrite poisoning, *Cichorium intybus*, guinea pig

Guinea pigs are placental mammals of the order Rodentia which holds places in human history that predates the European acquaintance with the small mammal and have been extensively used in immunology, nutrition, otology, and genetics and infectious studies (Clark and Olfert 1986). The diet of guinea pigs requires high amounts of amino acids and adequate amounts of fiber. These can be met by supplementing legumes such as Alfalfa, *Cichorium intybus*, forages (e.g., grass), hay, and vegetables (e.g., lettuce, spinach, carrot, tomato, cantaloupe), which can be gradually introduced in the diet to prevent digestive upsets (Banks 1989).

Nitrate occurs naturally in soil containing nitrogen-fixing bacteria, decaying plants, septic system effluent, nitrogenous fertilizers, air-borne nitrogen compounds emitted by industry, automobiles and animal manure (Deena et al 2006). The acute form of nitrate poisoning manifests itself primarily as methemoglobinemia, a blood disorder leading to hypoxia and sudden death (Radostits 2009). In this article, we present the incidence of nitrate-nitrite toxicity in guinea pigs (*Cavia porcellus*), which to the best of our knowledge, is the first report of accidental nitrate-nitrite toxicity in these animals due to the ingestion of the green leaves of a woody, perennial herbaceous forage crop called common chicory or *C. intybus* which was applied excessive levels of fertilizer i.e urea.

A total of 88 guinea pigs (25 males and 63 females) of 3-4 months old with an average body weight of 250±30 g were procured from Kings Institute of Preventive Medicine, Chennai, Tamil Nadu, India. The animals were maintained at the Laboratory Animal Division of Institute of Veterinary Preventive Medicine (IVPM), Ranipet, Animal Husbandry Department, Tamilnadu, India for vaccine production and validation. The animals were fed under an intensive method in a deep litter system. The guinea pigs were fed twice a day with Bengal gram at 20 g per guinea pig and wheat bran at 10 g per guinea pig in the morning at 8.00am. In the afternoon at 3.00pm leaves of *C. intybus* (120 g) and carrot (20 g), which were procured locally and daily, were given to each guinea pig. Reverse osmosis (RO) water was supplied ad libitum to all the guinea pigs. Spontaneous death in 28 out of 40 guinea pigs that were housed together in 1 compartment occurred after 30 minutes of feeding of C intybus leaves in the afternoon. Following this incident, the feeding of the remaining 48 guinea pigs housed in the adjacent compartment was stopped immediately. Among the 40 guinea pigs, 28 (70%) suffered from spontaneous death and showed bloody discharge from the nose, mouth and anus, cyanotic mucous membrane, mydriasis, subnormal body temperature, and a bloated abdomen. Necropsy was performed in all the 28 guinea pigs, and tissue samples from the liver, kidney, spleen, heart, brain, lung, trachea, esophagus, stomach and intestine were collected in 10% formalin, and processed routinely for histopathological examination. Heart blood swabs were collected for bacteriological studies. About 500 g of feed samples (*C. intybus* leaves) and a loop of small intestine of about 15 cm length were sent to the Central Animal Feed and Food Residue Laboratory (CAFFRL), TANUVAS, Madhavaram, Chennai, India for toxicological analysis. At the CAFFRL, the concentration of nitrate-nitrite was determined by measuring absorbance at 500 nm using a previously reported flow-injection spectrophotometric method (Andrade et al 2003). Necropsy revealed bloody discharges from all natural orifices (Figure 1) which clotted on exposure to air, chocolate brown discoloration of the blood, and haemorrhages in the gastrointestinal mucosa, heart, lung and kidney. Histopathological examination revealed mild focal hemorrhage in the epicardium of heart, multifocal hemorrhage in the lungs and diffuse mild tubular nephrosis of the kidney, mild lymphoid depletion in the spleen. In liver, hepatocytes revealed diffuse vacuolar changes (Figure 2), degeneration, mid zonal necrosis (Figure 3). Intestines revealed infiltration of mono nuclear cells in the lamina propria (Figure 4-5), and fusion of villi (Figure 4). No bacterial growth was detected from the heart blood swab. Toxicological analysis of the feed samples of *C. intybus* leaves showed 2.1 g% nitrate, 1.0 g% nitrite, and 80 ppb citrinin. The intestinal loop had 10 ppm of nitrate and 1 ppm of nitrite.

The present findings in guinea pigs are, to the best of our knowledge, the first report regarding the ingestion of *C. intybus* leaves that caused nitrate-nitrite toxicity because of their high amounts of nitrate under an excessive level of nitrogen fertilization i.e Urea. The toxic effects of nitrate due to accidental ingestion have been studied in different species including cattle (Ozmen et al 2003), cattle and sheep (Mckenzie et al 2004), fresh water invertebrates (Camargo et al 2005) and alpaca (Mckenzie et al 2009). Moreover, the toxic effects of nitrites have been investigated in 3 fresh water invertebrates (Alonso and Camargo 2006). On the other hand, induced nitrate toxicity has been extensively reported in wild animals such as herring gulls and ring-billed gulls (David 1986), New Zealand White Rabbit (Gilman et al 1998) and in laboratory animals such as Sprague-Dawley rats (Gilman et al 1998a) and guinea pigs (Stoewsand et al 1973).

The predominant necropsy finding of chocolate brown and coagulated blood from the natural orifices of the guinea pigs that succumbed is in accordance with the findings of nitrate toxicity in cattle (Ozmen et al 2003). In the determination of nitrate-nitrite toxicity from green leafy vegetables (Kaushalya et al 1989) and forages (Cathy et al 1995) it was found that the spectrophotometric method recommended by the Association of Official Analytical Chemists was the most effective method. This method also proved to be highly effective in making a confirmative diagnosis regarding the nitrate-nitrite toxicity in the present guinea pigs.

Individual animals may vary in their susceptibility to nitrate toxicity. The level of nitrate toxicity depends on the extent of conversion of nitrate to nitrites, and the process of nitrite formation is highly variable. One animal, may for instance, consume highly toxic levels of nitrates but not be poisoned because of poor conversion to nitrite, and the nitrites may fail to concentrate. Other animals may consume less amounts of nitrates, but convert the consumed nitrates rapidly to nitrite, and thus succumb to death

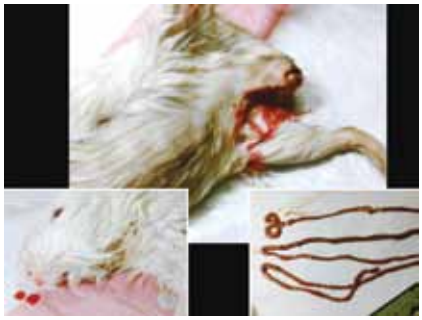

Figure 1. Haemorrhages in the anus, nostrils (insitu) and intestines (insitu) in a guinea pig.

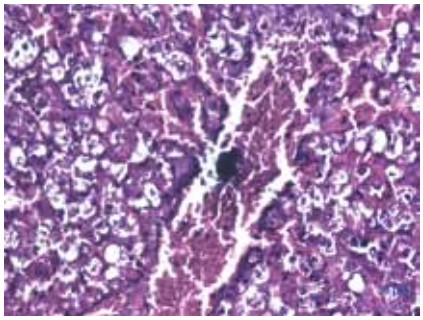

Figure 2. Liver showing diffuse vacuolar changes of hepatocytes (H&E).

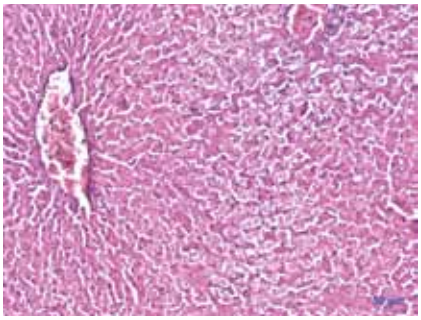

Figure 3. Liver showing mid zonal necrosis (H&E).

by poisoning (Olson et al 2002). This was similarly observed in the present case of ingestion by guinea pigs of *C. intybus* leaves which contained high amounts of nitrate from overfertilization. In this incident, 28 guinea pigs died out of the 40 guinea pigs exposed to nitrate-nitrite toxicity, indicating possible variation in their susceptibility to nitrate-nitrite poisoning.

In conclusion, the green leaves of *C. intybus*, which contained 2.1 g% nitrate, 1.0 g% nitrite, and 80 ppb citrinin from overfertilization, caused acute nitrate-nitrite toxicity in guinea pigs and resulted in up to 70% mortality. Extreme care should be observed when feeding overfertilized *C. intybus* to guinea pigs. The risk of poisoning can also be reduced by having feeds analyzed for nitrates when in doubt.

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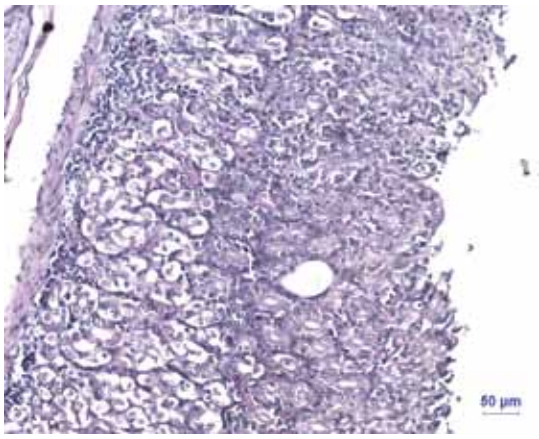

Figure 4. Intestine showing infiltration of mononuclear cells in the lamina propria and fusion of villi (H&E).

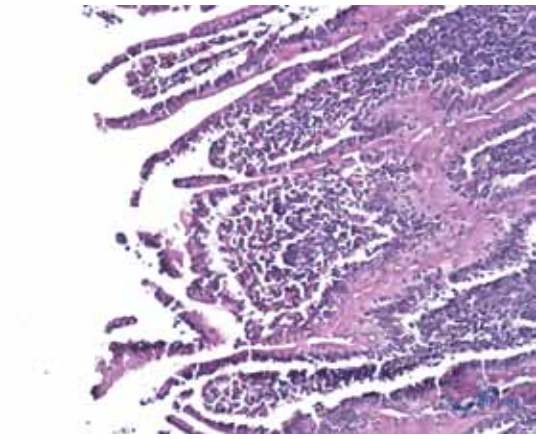

Figure 5. Infiltration of mononuclear cells in the intestine (H&E).

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