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ACUTE SYNTHETIC AZO DYE INTOXICATION IN A CAT – CLINICAL CASE

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ABSTRACT

The paper describes a case of acute intoxication in a cat that had ingested a tree fern – *Asparagus virgatus* that was known to be treated with a green synthetic azo dye. Clinical examination and paraclinical analyses revealed restlessness, tachycardia, tachypnea, cyanosis of mucous membranes, oxidative damage, and methemoglobinemia. Based on the history, the clinical signs and laboratory findings, indicative of azo dye intoxication and response to treatment, the diagnosis of acute synthetic azo dye intoxication was made.

Key words: intoxication, synthetic, azo, dye, cat.

Introduction

The synthetic dyes used by the commercial establishment to enhance the color of the plant are sourced from ROBERT KOCH Industries Inc. These green dyes are made up of different types of Acid Yellow, Acid Blue, and Direct Blue, which are azo dyes. Various industries make extensive use of synthetic azo dyes. (Benkhaya S. *et al.*, 2020). Azo dyes are identified by the presence of the (–N=N–) functional group, which connects two identical or dissimilar alkyl or aryl radicals, whether they are symmetrical or not. (McLaren K., 1983).

The main biotransformation products of azo dyes are aromatic amines. (Chequer FMD *et al.*, 2011). Azo dyes are often converted into toxic aromatic amines, such as benzidine, 4–aminobiphenyl, and 2–naphthylamins known for their potent mutagenic, carcinogenic, and hemotoxic effects when absorbed from the skin, gut, and respiratory tract. (Siddiqui, S.I. *et al.*, 2023).

The development of cancer due to aromatic amines is initiated by the process of N–hydroxylation in the liver, followed by subsequent glucuronidation. (Chinthakindi S., Kannan K., 2022). Bladder cancer is relatively common in cats, and a deficiency in the glucuronide conjugation pathway is thought to play a role in its development as a result of chronic exposure to aromatic amines.

Aromatic amines (AAs) are efficiently metabolized in the liver, yielding metabolites that undergo redox cycling. Metabolic attacks on AAs primarily involve oxidation of the N atom (N–oxidation) and oxidation of the carbon in the aromatic ring (C–oxidation). Amine hydroxylation and esterification are key initial steps in their metabolic activation, contributing to the formation of toxic agents. (Siddiqui, S.I. *et al.*, 2023).

The amines oxidize the heme iron of hemoglobin from Fe (II) to Fe (III), blocking oxygen binding. as a result, distinct acute symptoms such as cyanosis of the mucous membranes, weakness, and dizziness become evident. (Øllgaard H., *et al.*, 1998). The N–hydroxylated metabolites of numerous aromatic amines engage in a co–oxidation process with oxy–hemoglobin (HbO2), resulting in the formation of methemoglobin (met–Hb). (Raghu G. *et al.*, 2016).

The manifestation of these deleterious effects was observed in the context of the clinical case.

Case Presentation

The patient was a 4–year–old female, mixed – breed cat, named Fabien, weighing 5.9 kg, with no previous medical history.

According to the owners, the cat had ingested a small quantity of tree fern (Asparagus virgatus) a few hours prior. The history revealed a single episode of vomiting, which included parts of the plant, as well as restlessness and lethargy. The owners observed the plant's vibrant coloration and subsequently ascertained from the florists the specific dye employed in its treatment.

Clinical examination was conducted using routine techniques.

Hematological and biochemical analyses were conducted by collecting a blood samples from the v. saphena lateralis using a 22 G needle. The complete blood counts (CBCs) on day 1 (D1), day 2 (D2), and day 3 (D3) were analyzed using a Mindray BC–288 Vet automatic blood counting analyzer. The biochemical profile on D1 was analyzed using an automatic analyzer VET–CHEM100. For the blood smear, an EDTA blood sample was used. It was fixed with methanol, stained with methylene blue, and then washed with distilled water. The smear was examined under an immersion microscope with a 100x1.25 zoom to detect oxidative changes in the erythrocytes.

The treatment included licensed veterinary and human drugs administered at appropriate doses and regimen.

Clinical examination revealed restlessness, weakness, cyanotic mucous membranes (Fig. 2), capillary refill time – 2.5 s., tachypnea (RR= 55bpm), tachycardia (HR= 230 bpm) and body temperature (38.9 °C). The palpation of abdominal cavity did not reveal any abnormalities.

The blood obtained for analysis was dark – brown in color, indicative of methemoglobinemia.

Figure 1: *Asparagus virgatus* treated with green azo dye (the dye is extracted onto thewhite sheet of paper).

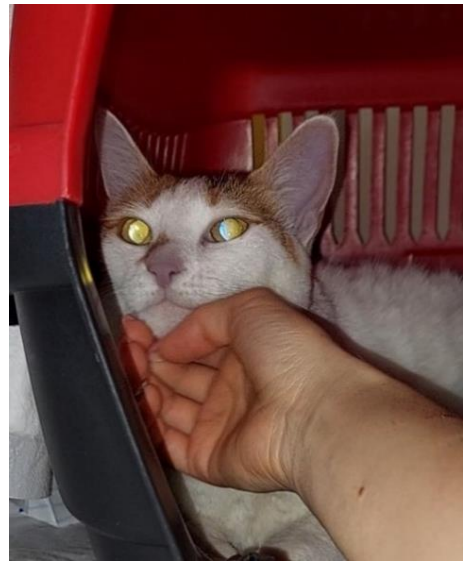

Figure 2: The cyanotic cat.

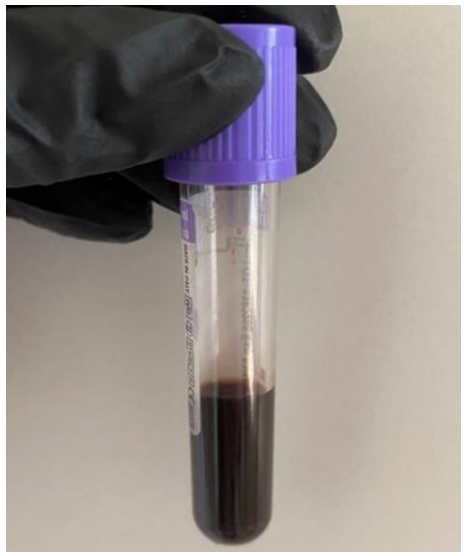

Figure 3: Obtained dark – brown blood sample.

Hematological analyses – the blood test results at D1, D2 and D3 are presented in Table 1, Table 2 and Table 3.

Table 1: Hematological indices at D1

Test	Result	Unit	Reference range cat
WBC	6.6	x 10^9 /L	5.5 - 19.5
LY	3.0	x 10^9 /L	0.8 - 7.0
Mon	0.2	x 10^9 /L	0.0 - 1.9
GR	2.6	x 10^9 /L	2.1 - 15.0
Ly %	45		12.0 - 45.0
Mon %	3.8		2.0 - 9.0
Gr %	47.5		35.0 - 85.0
RBC	9.99	x 10^{12}/L	4.60 - 10.00
Hgb	114	g/L	93 - 153
Hct	43.3	%	28.0 - 49.0
MCV	47.3	fl	39.0 - 52.0
MCH	L 12,7	pg	13 - 21
MCHC	L 293	g/L	300 - 380
Plt	147	x 10^9 /L	100 - 514
Eos %	2.1		

Table 2: Hematological indices at D2

Test	Result	Unit	Reference range cat
WBC	5.5	x 10^9 /L	5.5 - 19.5
LY	2.7	x 10^9 /L	0.8 - 7.0
Mon	0.2	x 10^9 /L	0.0 - 1.9
GR	2.6	x 10^9 /L	2.1 - 15.0
Ly %	H 48.7		12.0 - 45.0
Mon %	2.6		2.0 - 9.0
Gr %	51.0		35.0 - 85.0
RBC	9. 88	x 10^{12}/L	4.60 - 10.00
Hgb	130	g/L	93 - 153
Hct	43.0	%	28.0 - 49.0
MCV	42.7	fl	39.0 - 52.0
MCH	L 12.9	pg	13 - 21
MCHC	300	g/L	300 - 380
Plt	183	x 10^9 /L	100 - 514
Eos %	3.8		

Table 3:.Hematological indices at D3

Test	Result	Unit	Reference range cat
WBC	8.5	x 10^9 /L	5.5 - 19.5
LY	2.3	x 10^9 /L	0.8 - 7.0
Mon	0.4	x 10^9 /L	0.0 - 1.9
GR	5.9	x 10^9 /L	2.1 - 15.0
Ly %	26.2		12.0 - 45.0
Mon %	5.5		2.0 - 9.0
Gr %	68.3		35.0 - 85.0
RBC	9. 67	x 10^{12}/L	4.60 - 10.00
Hgb	144	g/L	93 - 153
Hct	45.7	%	28.0 - 49.0
MCV	47.3	fl	39.0 - 52.0
MCH	14.8	pg	13 - 21
MCHC	315	g/L	300 - 380
Plt	127	x 10^9 /L	100 - 514
Eos %	2.1		

On D1 and D2, there was a decreased mean corpuscular hemoglobin (MCH) and on D1, there was also a decreased mean corpuscular hemoglobin concentration (MCHC).

Additionally, Heinz bodies formation was detected on the blood smear.

The hematological alterations, including decreased Mean Corpuscular Hemoglobin(MCH) and decreased Mean Corpuscular Hemoglobin Concentration (MCHC), along with the formation of Heinz bodies and the presence of methemoglobinemia, are indicative of oxidative damage caused by the toxic intermediates of azo dye.

Biochemical analysis yielded unremarkable results, possibly attributed to the prompt response of the owners.

On D2, there was a slight increase in lymphocytes, suggesting an immune response to the toxic substance. However, on D3, the complete blood count showed normal values.

Based on the history, clinical signs, laboratory findings and response to the treatment acute synthetic azo dye intoxication was diagnosed.

The owners reported that the cat consumed a small quantity of the plant and did not eat the fern's berries, which are known to contain the highest levels of toxic saponins.

Hence, we posit that the primary origin of toxicity and the majority of the symptoms, possibly excepting the initial vomiting, stem from the synthetic azo dye.

The main focus of the treatment was directed toward addressing the effects of the dye.

The cat was hospitalized in the clinic for two days and the treatment protocol included:

- 0.9 % NaCl solution –3 ml/kg/h i.v. constant rate infusion;
- Activated charcoal – 3 g/kg p.o q2 h – three times;
- Vitamin C – 30 mg/kg i.v q8 h;
- N – acetylcysteine – 140 mg/kg p.o , followed by 70 mg/kg p.o q4 h;
- Ornipural® (Betaïne, Arginine, Ornithine, Citrulline) – 3ml/ kg i.v q24 h;

Within two hours of starting treatment and approximately one hour after the initial intake of N–acetylcysteine, the cat's condition significantly improved. The heart and respiratory rates returned to normal, and the mucous membranes began to regain a normal color. N–Acetyl Cysteine was administered within 8 hours of toxin ingestion.

On the second day, the patient's overall well–being was enhanced, and the cat started to eat.

The mucous membranes appeared normal and the cat was discharged from the clinic. By the third day, the patient's condition had completely normalized.

The dietary supplement Zentonil Advanced® (S–Adenosylmethionine, Silybin) was prescribed at a dose of 1/2 tablet p.o q12 h for 10 days.

After ten days, a follow–up examination of the patient was performed, and control blood tests were conducted, which showed no abnormalities.

Discussion

Numerous toxic substances in the environment pose a threat to pets' health. Azo dyes that contain toxic aromatic amines are widely used in a variety of industries, including cosmetics, finished textiles, and plant dyeing.

The full extent of synthetic azo dyes toxicity remains unstudied, which complicated the diagnosis in this particular case. However, the diagnosis was determined based on the provided information that the animal had ingested a plant treated with synthetic azo dyes, the clinical signs, hematological alterations indicative of azo dye intoxication, and the response to treatment aided in confirming the diagnosis.

The primary methods of detoxifying metabolism for azo dyes and aromatic amines involve oxidative processes, which encompass ring hydroxylation and the conjugation with glucuronide. (Øllgaard H., *et al.*, 1998).

Cats exhibit a relatively slow or even limited ability to form glucuronides with many compounds.

This is attributed to their possession of fewer isoforms of the enzymes responsible for mediating this conjugation, known as glucuronyl transferases. (Allen AL., 2003). The relative deficiency of glucuronide conjugation pathway results in more drugs being conjugated to sulfates; however,

the sulfation pathway has a finite capacity, which is also lower in cats than in other species (Aronson LR., Drobatz K., 1996).

In cats, the deficiency in the glucuronide conjugation pathway can lead to the accumulation of toxic metabolites derived from the metabolism of aromatic amines.

The treatment protocol, including N–acetylcysteine (NAC), is aimed at eliminating toxic metabolites, combating oxidative stress, preventing liver damage and carcinogenic effects of toxic metabolites.

NAC is a synthetic derivative of the endogenous amino acid L–cysteine and a precursor of glutation, which is synthesized and maintained at high concentrations in all cells, is one of the mechanisms by which cells protect themselves from oxidative stress.

More recently, improved knowledge of the mechanisms by which N–acetylcysteine (NAC) acts has expanded its clinical applications. It plays a key role in the detoxification of many compounds such as aromatic amines. (Raghu G. *et al.*, 2021).

We consider that the prompt action by the owners and timely treatment including N – acetylcysteine (NAC) resulted in the cat's swift recovery.

References

1. Allen A.L. (2003). *The diagnosis of acetaminophen toxicosis in a cat*. Can Vet J. Volume 44, Number 6, 509–510.
2. Aronson L.R., Drobatz K. (1996). *Acetaminophen Toxicosis In 17 Cats*. Journal of Veterinary Emergency and Critical Care, Volume 6, Number 2, 65–69.
3. Benkhaya S., M'rabet S., El Harfi A. (2020). *A review on classifications, recent synthesis and applications of textile dyes*. Inorganic Chemistry Communications, Volume 115, Number 107891.
4. Chequer F.M.D, Dorta D.J, de Oliveira D.P. (Chap. 2). *Azo dyes and their metabolites: does the discharge of the Azo dye into water bodies represent human and ecological risks?* In: Advances in treating textile effluent, London, InTech.
5. Chinthakindi S., Kannan K. (2022). *Urinary and fecal excretion of aromatic amines in pet dogs and cats from the United States*. Environment International, Volume 163, Number 107208.
6. McLaren K. (1993). *The Colour Science of Dyes and Pigments*. Bristol, UK: Adam Hilger Ltd. 1st Edition
7. Øllgaard H., Frost L., Galster J., Hansen O.C. (1998). *Survey of azo–colorants in Denmark: Consumption, use, health and environmental aspects*. 221 Danish Environmental Protection Agency, Miljøprojekt 509, 217–215.
8. Raghu G., Berk M., Campochiaro PA, Jaeschke H., Marenzi G., Richeldi L., Wen FQ., Nicoletti F., Calverley P.M.A. (2021). *The Multifaceted Therapeutic Role of N–Acetylcysteine (NAC) in Disorders Characterized by Oxidative Stress*. Curr. Neuropharmacol. Volume 19, Number 8, 1202–1224.
9. Siddiqui, S.I.; Allehyani, E.S.; Al–Harbi, S.A.; Hasan, Z.; Abomuti, M.A.; Rajor, H.K.; Oh, S. (2023). *Investigation of Congo Red Toxicity towards Different Living Organisms: A Review*. Processes 2023, 11, 807