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Case Study

Possible Acute Poisoning by Sinapis arvensis in Sheep: Clinical, Laboratory and Necropsy Findings

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Abstract

An outbreak of possible acute poisoning by *Sinapis arvensis* occurred in a flock of 50 fat-tailed sheep located in the Semnan province of Iran. *Sinapis arvensis* is an annual or winter annual plant of the genus *Sinapis* in the family Brassicaceae, commonly known as field mustard, wild mustard or charlock. The poisonous constituents are volatile oil of mustard, the alkaloid sinapin, and the alkaloidal glucoside sinalbin. The flock was grazing in land containing high amounts of wild mustard (Sinapis arvensis) in late spring. Seven sheep (aged between 1 and 5 years) died within approximately 3 days. The affected animals displayed signs of depression, reluctance to move, tachycardia, tachypnea, mucoid and hemorrhagic nasal discharges, pale conjunctiva, ataxia, abdominal pain, bruxism, and anorexia. Rectal temperature in these animals was normal to high (39-41.5 °C). Ruminal movements were reduced (1-2/min). Serum biochemical levels in affected sheep showed marked increases of blood urea nitrogen (BUN), Gamma glutamyl transferase (GGT), magnesium (Mg), and phosphorous (P) and a marked decrease in calcium (Ca). In urinalysis, marked hemoglobinuria and proteinuria were observed. Necropsy findings included congestion in lungs and hemorrhage on the epicardial and endocardial heart surfaces, on the surface and medulla of the kidneys, and abomasal mucosa. The liver was also congested with a nutmeg pattern. Rumen contents included digested materials and large quantities of seeds and stems of wild mustard. For the first time, our findings confirmed wild mustard toxicosis in sheep in Iran.

Keywords: wild mustard, *Sinapis arvensis*, sheep, Semnan, possible poisoning

Intoxication Aiguë Possible par *Sinapis arvensis* chez les Moutons: Résultats Cliniques, de Laboratoire et de la Nécropsie

Résumé: Une épidémie d'intoxication aiguë possible par *Sinapis arvensis* s'est produite dans un troupeau de 50 moutons à queue grasse situé dans la province de Semnān en Iran. *Sinapis arvensis* est une plante annuelle ou annuelle d'hiver du genre *Sinapis* de la famille des Brassicaceae, communément appelée moutarde, moutarde des champs ou charlock. Les constituants toxiques sont l'huile volatile de moutarde, l'alcaloïde sinapine et le glucoside alcaloïde sinalbine. Le troupeau paissait sur des terres contenant de grandes quantités de moutarde sauvage (*Sinapis arvensis*) à la fin du printemps. Sept moutons (âgés de 1 à 5 ans) sont morts en 3 jours environ. Les animaux affectés présentaient des signes de dépression, de réticence à bouger, de tachycardie, de tachypnée, d'écoulement nasal mucoïde et hémorragique, de conjonctive pâle, d'ataxie, de douleurs abdominales, de bruxisme et d'anorexie. La température rectale chez ces animaux était de normale à élevée (39-41,5 °C). Les mouvements du rumen ont été réduits (1-2/min). Les niveaux biochimiques sériques chez les moutons affectés ont montré des augmentations marquées de l'azote uréique du sang (BUN), de la Gamma glutamyl transférase

(GGT), du magnésium (Mg) et du phosphore (P) et une diminution marquée du calcium (Ca). Dans l'analyse d'urine, une hémoglobinurie et une protéinurie marquées ont été observées. Les résultats de la nécropsie comprenaient une congestion des poumons et une hémorragie sur les surfaces cardiaques épicardique et endocardique, sur la surface et la moelle des reins, et la muqueuse de la caillette. Le foie était également congestionné avec un motif de noix de muscade. Le contenu du rumen comprenait des matières digérées et de grandes quantités de graines et de tiges de moutarde sauvage. Pour la première fois, nos résultats ont confirmé la toxicose de la moutarde sauvage chez les moutons en Iran.
Mots-clés: moutarde sauvage, *Sinapis arvensis*, mouton, Semnān, intoxication possible

1. Introduction

Sinapis arvensis is an annual or winter annual plant of the genus *Sinapis* in the family Brassicaceae. It is commonly known as field mustard, wild mustard, or charlock. Wild mustard is an aggressive weed indigenous throughout most of the temperate regions of Europe, Asia Minor, southwest Asia, and North Africa. Wild mustard seed is similar in size and shape to canola seed (1). The height of *sinapis arvensis* ranges between 20 and 80 centimeters, but under optimal conditions, it can exceed one meter. The stems are straight, branched, and striated with coarse spreading hairs, especially near the base. The substance responsible for toxicosis is sinigrin, which, in the presence of the enzyme myrosinase, is converted to glucose, allylisothiocyanate (mustard oil), and potassium hydrogen sulfate. Moreover, mustards occasionally contain toxic concentrations of nitrate that may complicate toxicosis. Sheep do not routinely consume mustard; however, when not having access to regular food, the animal may eat this plant. The objective of this report was to describe an outbreak of acute poisoning by *Sinapis arvensis* in a flock of sheep from Iran based on clinical signs, laboratory and necropsy findings (2).

2. Material and Methods

2.1. Clinical Presentations

An outbreak of acute *Sinapis arvensis* poisoning occurred in a flock of 50 fat-tailed sheep located in the Semnan province of Iran. Grazing in a land containing large amounts of wild mustard and minute amounts of weeds such as ryegrass caused the poisoning. Within approximately 3 days, 7 sheep had died. The affected animals displayed signs of depression, reluctance to move, tachycardia, tachypnea, mucoid and hemorrhagic nasal discharges, pale conjunctiva, ataxia, abdominal pain, bruxism, and anorexia. The size of the lymph nodes was normal. Rectal temperatures in these animals ranged from normal to high (39-41.5 °C), and ruminal movements were reduced (1-2/min). The sheep tended not to urinate or defecate easily, and urine was very concentrated. Diarrhea was observed in some animals. Lungs were congested and emphysematous. Foamy materials were observed in the trachea. Petechial to ecchymosis hemorrhages involved the epicardial and endocardial heart surfaces and abomasal mucosa. Alimentary tract inactivity caused gallbladder distension with viscid bile. Intestines were congested, and black areas on the surface of the small intestine were observed. Rumen contents included digested materials, large quantities of wild mustard seeds and stems, and small amounts of undigested barley. The pH of the rumen fluid was 5.5.

3. Results

3.1. Diagnostic Testing

Blood samples were collected from five intoxicated sheep for hematology and serum biochemistry analysis (Table 1). An increase in glucose was observed in Case 2 (up to 400 mg/dl), but the cause of this increase could be severe liver damage or sepsis or errors in sample measurement. Total leukocyte, neutrophil, and lymphocyte counts and total protein and fibrinogen concentrations were in the normal range, but packed cell volume (PCV) was reduced. Serum analysis

revealed marked hypocalcemia, relatively high magnesium and phosphorous, and high activity of serum GGT. Necropsies were performed on all dead animals. All mucous membranes were congested. The liver was also congested with a nutmeg pattern. Kidneys were inflamed and had visible pale areas, and petechial hemorrhages were evident on the surface and medulla (Figure 1, Figure 2, Figure 3, and Figure 4). The urine strip analysis results demonstrated mild hemoglobinuria (+) and proteinuria (+). Urine was analyzed and revealed marked hemoglobinuria and proteinuria. Although the animals were not suffering from dehydration, the urine specific gravity was high (Table 2).

Table 1. Hematological and *serum biochemical parameters results*

	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Normal range***
PCV (%)	24	43	24	25	22	27-45
Total WBC (Cell/µL)	8700	18000	9950	1020	15000	4000-12000
Neutrophils (Cell/µL)	6438	13680	4975	388	9000	700-6000
Lymphocytes (cell/µL)	2262	4320	4179	632	6000	2000-9000
Eosinophil (Cell/µl)	-	-	796	-	-	0-1000
Heinz bodies	-	-	-	-	-	
Blood parasites	-	-	-	-	-	
Total protein (g/dl)	6.0	7.9	7.4	6.5	6.0	6.0-7.9
Fibrinogen (mg/d)	300	500	300	450	300	200-500
Albumin (g/dl)	2.4	3.3	2.8	-	-	2.4-3.0
BUN (mg/dl)	32.5	33.1	-	3.87	0.26	8.0-20
Creatinine (mg/dl)	1.4	1.42	0.5	0.96	0.90	1.2-1.9
Glucose (mg/dl)	27.8	400	35.5	-	-	50-80
Cholesterol (mg/dl))	33.6	50.6	38.5	-	-	43-103
Total bilirubin (mg/dl)	0.3	0.5	0.17	-	-	0.1-0.5
ALT (iu/l)	38.6	24.8	12.2	-	-	22-38
AST (iu/l)	695	217	106	-	-	60-280
ALP (iu/l)	106	78.3	117	-	-	70-390
GGT (iu/l)	214	83	44.6	63.05	38.4	20-52
Mg (mg/dl)	2.44	3.27	3.4	-	-	2.2-2.8
Ca (mg/dl)	11.3	13.5	12.5	5.24	5.20	11.5-13.0
P (mg/dl)	9.84	6.68	6.3	21.44	22.34	5.0-7.3

* Reference: Veterinary Medicine, 10th edition. (F)

Table 2. Urinalysis results

Urine parameters	**Amount**
Color	Yellow
Hgb	250
Urobilinogen	0
T Bilirubin	+2
Protein	500
Nitrite	0
Ketone Body	5
Asc.A.	10
Glu	0
PH	6
SPG	1.025
Cast	Granular Cast Waxy Cast RBC Cast

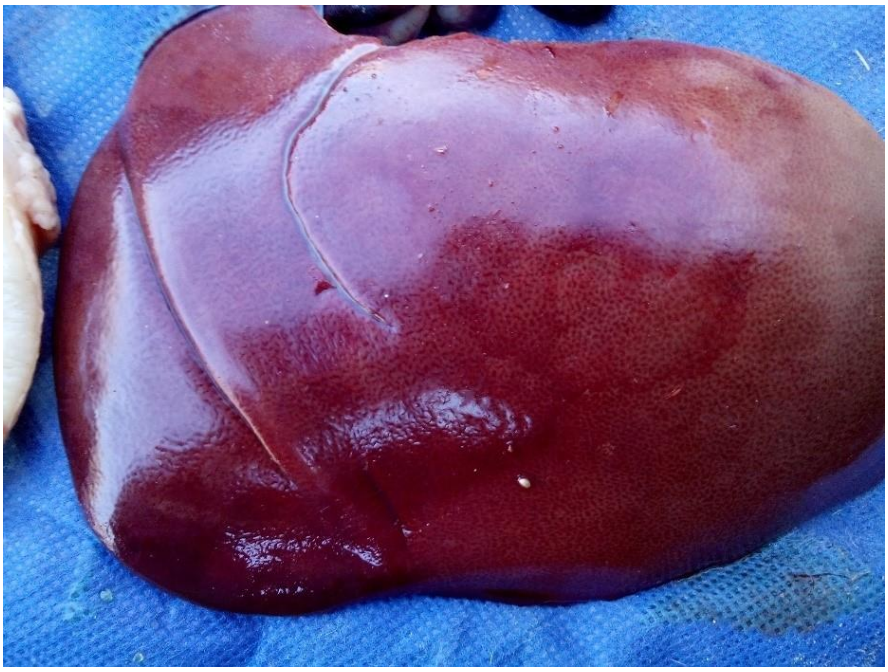

Figure 1. Congestion in liver

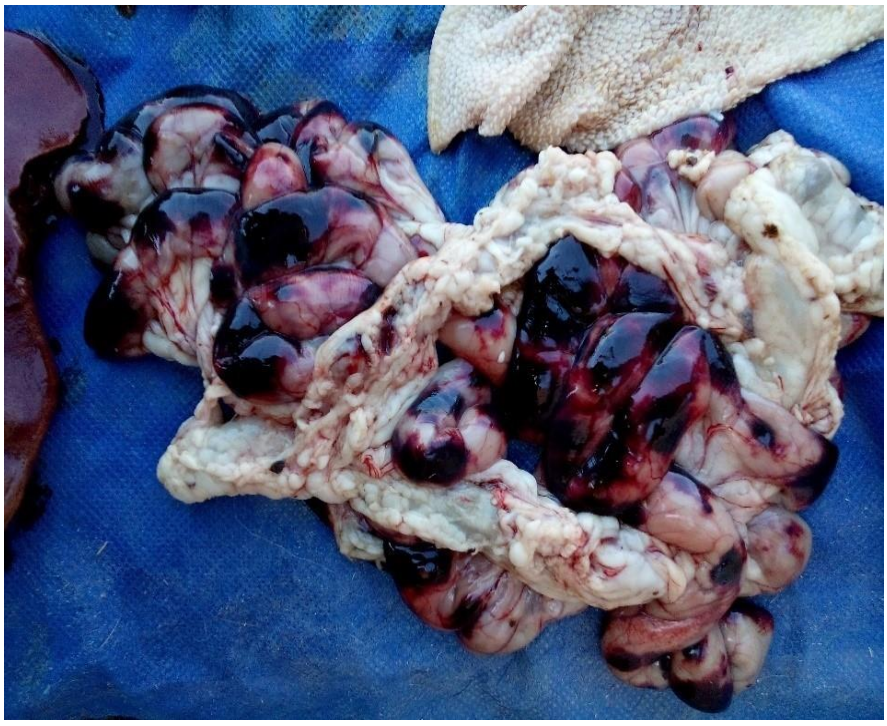

Figure 2. Intestines containing hemorrhagic fluids; congested and dark areas visible on intestines

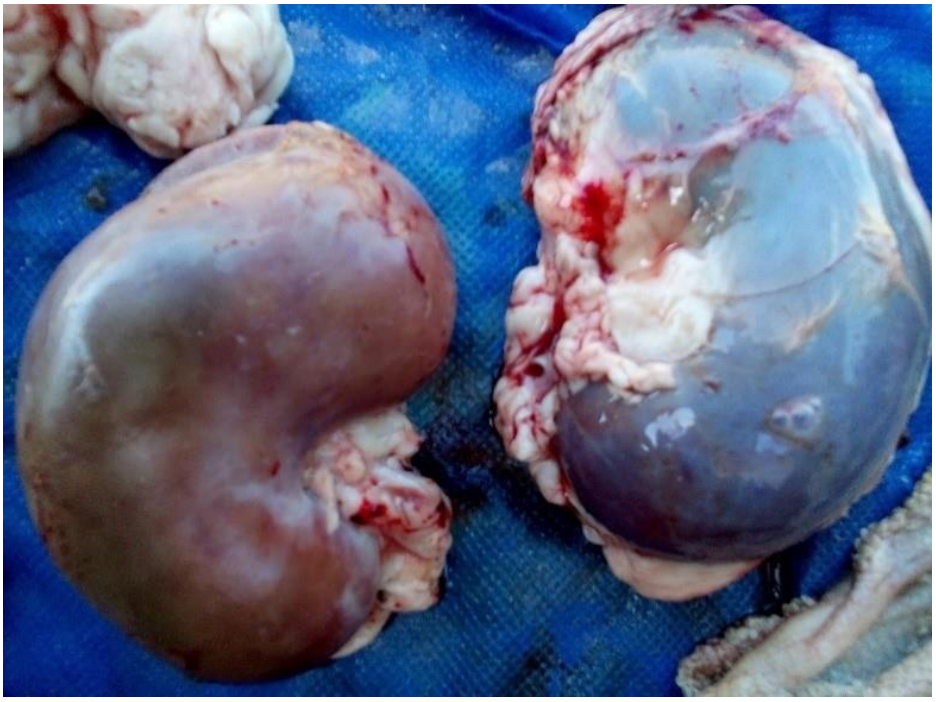

Figure 3. Inflamed kidneys with visible large and pale areas

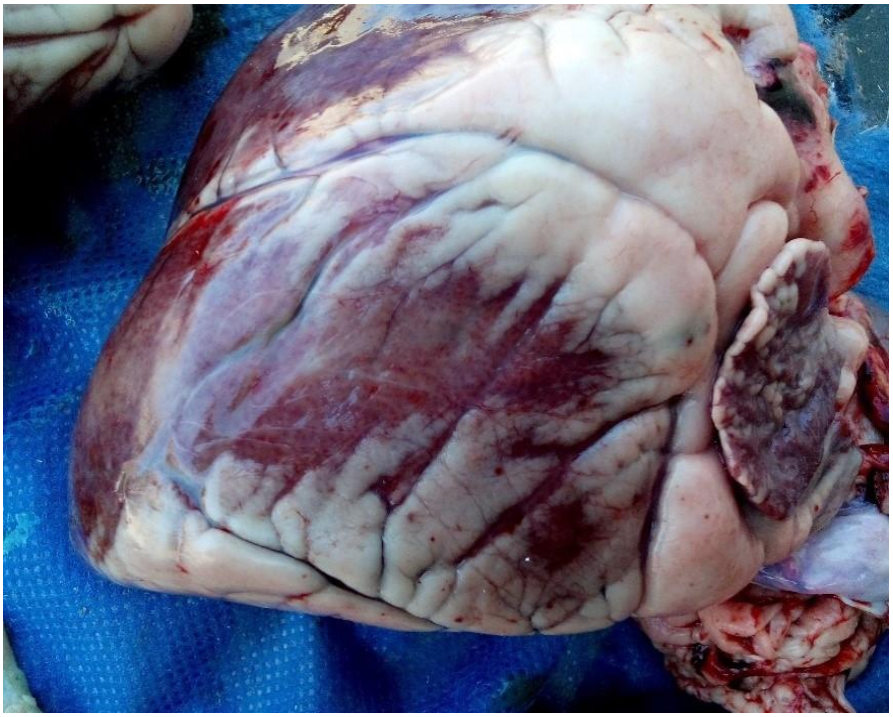

Figure 4. Petechial hemorrhage on epicardium

4. Discussion

Diagnosis of *Sinapis arvensis* poisoning was based on the existence of the plant around where the sheep grazed, clinical signs, and post-mortem findings such as large amounts of digested materials and wild mustard seeds and stems in the rumen of the dead animals. Wild mustard seed poisoning in cattle was previously reported by Gwatkin, Moynihan (2). Mustard poisoning receives little attention, because only the seeds are harmful, and as a rule, animals obtain the poison only when the seeds are combined with concentrated feeding stuffs. Horses and cattle are affected to some extent, but as might be expected, pigs suffer the most injury. The poisonous constituents are volatile oil of mustard, the alkaloid sinapin, and the alkaloidal glucoside sinalbin (2). Brassica spp. are part of the family Cruciferae, or the mustard group of plants, which often contain a sulfonated oxime group combined with glucose in the form of glycosides, referred to as glucosinolates. These in Brassica spp. and related plants are used as fodder (3-5). Glucosinolates are present in the vegetative parts of these plants and in much higher concentrations in the seeds. Glucosinolates are hydrolyzed by an enzymatic system of thioglucosidases and myrosinase in the parenchymal tissues of the plant. Products of hydrolysis include glucose, hydrosulfuric acid, and an aglycone containing nitrogen and sulfur. The last product is unstable and hydrolyzed to isothiocyanate and organic nitrile. Glucosinolates are irritants for the alimentary tract mucosa and goitrogen. Also, there is a positive correlation between cruciferous plants (Brassica spp.) (6-8) and polio encephalomalacia in ruminants, e.g. in rape blindness (2, 9). Iodine deficiency, which includes both primary and secondary conditions, results from the presence of glucosinolates in the diet (4). Brassica spp. also contain S-methyl-L-cysteine sulfoxide (SMCO). SMCO is a rare sulfur-containing α amino acid which occurs in some genera of plants in the family Brassicaceae. Its metabolic products in ruminants, dimethyl disulfide produced by ruminal bacteria from SMCO, are associated with serious hemolysis and anemia (10). Outbreaks and heavy morbidity and case fatality rates occur when mature plants are grazed. The green parts of the plant are usually involved in outbreaks. The SMCO content of forage brassicas increases as the plant matures (10, 11). The plants may also contain significant quantities of cyanogenetic glycosides but are rarely associated with cyanide poisoning. Nitrate and nitrite poisoning in feeding have also been recorded (10). The important factors in most outbreaks are the maturity of the crop, the amount eaten, plant stress including drought and overcrowding, and high sulfate diets. The plants are harmless until the cells of the seeds are moistened, crushed, and acted on by ruminal microflora. The commonest and most serious cases of poisoning occur in animals fed rapeseed or rapeseed meal (9-11). The clinical pathological changes caused by wild mustard poisoning in sheep confirmed renal damage associated with a renal tubular lesion. The glomerular leakage or lack of proximal tubular reabsorption of protein or both may cause increased urinary protein loss to develop. The presence of moderate numbers of granular and waxy casts in the urine sediment confirmed severe tubular damage. Histological and clinical pathological investigations were mainly consistent with mild to moderate, reversible injury to the hepatobiliary. However, increased serum GGT concentration may originate from different tissues, but notable elevations in activity in the serum are primarily observed in liver disease and indicate hepatobiliary disease associated with cholestasis (12, 13). Hepatobiliary disease may develop primarily because of toxic damage to the hepatocytes, or secondary to hypoxia or metabolic conditions that developed during renal failure (14). Hypocalcemia and hypophosphatemia also indicated tubular damage, although it would have been better proved if fractional excretion of phosphor (FE_P) and calcium (FE_{Ca}) had been calculated in this study. FE_{Ca} as well as sodium (FE_{Na}) can be used to distinguish between prerenal lesions and tubular necrosis. FE_{Ca} below 1% is indicative of prerenal lesions, while FE_{Ca} above 1% can be seen with tubular necrosis (15, 16). Unfortunately, information on toxin levels in the rumen

is not complete; based on herd losses after grazing in the pasture and ruling out other possible poisonings, however, poisoning with wild mustard is the most likely diagnosis. For treatment, the sheep were removed from the pasture. Supportive treatment included intravenous injection of solutions containing 3.3% dextrose and 0.3% NaCl. The affected sheep showed signs of recovery within 5-6 days after treatment, and no deaths were observed.

Authors' Contribution

Study concept and design: M. K.
Acquisition of data: E. Sh.
Analysis and interpretation of data: M. A. H.
Drafting of the manuscript: H. E. Ch.
Critical revision of the manuscript for important intellectual content: A. J. V.
Statistical analysis: M. K.
Administrative, technical, and material support: M. K.

Ethics

All procedures performed in studies involving animals were in accordance with the ethical standards of the Semnan University, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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