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Intoxication due to the consumption of huinar (*Melochia pyramidata*) in bovine of Colima, Mexico

Bovinos intoxicados por *Melochia pyramidata* en Colima, México

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ABSTRACT

With the purpose of describing the pathological conditions of bovines intoxicated by consuming huinar (*Melochia pyramidata*) in Colima, Mexico, there are three cases of animals with signs of incoordination, ataxia, and paralysis of the posterior train, prostration and death, suspects of bovine paralytic rabies. Complementary studies of hematology, necropsies, histopathology, and immunofluorescence were performed and *M. pyramidata* plants were collected for the detection of alkaloids. Leukocytosis was detected by neutrophilia and normocytic normochromic anemia. The macroscopic findings were the presence of edema in multiple organs. Histopathologically the brain, cerebellum, spinal and oblongata cord, as well as peripheral nerves, presented axonal degeneration and areas of demyelination. In histological test without evidence of lesions, negative to rabies virus in immunofluorescence test and the presence of alkaloids was detected in *M. pyramitada*. Therefore, the consumption of alkaloids through *Melochia pyramidata* causes nervous disease in cattle that helps in the differential diagnosis of bovine paralytic rabies. Finally, the description of this case can help identify the toxic flora of western Mexico.

Keywords: toxic plants, huinar, Melochia pyramidata, cattle, toxicity.

RESUMEN

Con el propósito de describir las condiciones patológicas de bovinos intoxicados por consumir huinar (*Melochia pyramidata*) en Colima, México, se presentan tres casos de animales con signos de incoordinación, ataxia, parálisis del tren posterior, postración y muerte, sospechosos de rabia paralitica bovina. Se realizaron estudios complementarios de hematología, necropsia, histopatología e inmunofluorescencia, además se colectaron plantas de *M. pyramidata* para determinar la presencia de alcaloides. Macroscópicamente se observó edema en múltiples órganos, luego, se detectó leucocitosis por neutrofilia y anemia normocítica normocrómica en sangre. Histopatológicamente el cerebro, cerebelo, médula oblonga y espinal, así como nervios periféricos, presentaron degeneración axonal y áreas de desmielinización. En prueba histológica sin evidencia de lesiones, negativo a virus rábico en prueba de inmunoflorescencia y se detectó la presencia de alcaloides en *M. pyramitada*. Por lo tanto, el consumo de alcaloides a través de *Melochia pyramidata* provoca enfermedad nerviosa en bovinos que ayuda en el diagnóstico diferencial de rabia paralitica bovina. Finalmente, la descripción de este caso puede ayudar a identificar la flora tóxica del occidente de México.

Palabras clave: plantas tóxicas, intoxicación, huinar, Melochia pyramidata, bovinos.

INTRODUCTION

Mexico has 197 million hectares, 27% of the surface is from tropical zones, which have livestock destined to the production of meat and dual purpose (meat-milk). The production system in the dry tropics is cow-calf, development in pasture, fattening in grazing and corral; while in the humid tropics the system is cow-calf (double purpose), fattening in grazing and in corral.

The production of bovine meat in our country is concentrated in Jalisco (14%), Veracruz (14%), Sonora and Tamaulipas (5%), Chiapas, Tabasco, Durango and Chihuahua (4%) and Colima ranks 26th nationally (SAGARPA, 2014). In spite of the importance of the cattle activity, the problematic associated to the intoxication of the cattle by vegetables, has been little studied in Mexico; and it is the cause of economic losses that seriously affect producers (diseases, physical defects, abortions and death).

Among the main chemicals that contain plants that intoxicate livestock are: alkaloids, glycosides (cyanogenic or saponins), irritating oils, organic acids, minerals (nitrates, selenium and molybdenum), resins or resinoids, phytotoxins and toxic principles that cause photosensitivity; some plants contain two or more toxic principles. The main species that contain toxic alkaloids for cattle are: Hierba ceniza (*Senecio longilobus*), Chilicote or colorín (*Erythrina flabeliformis*), Lupinos (*Lupinus sparsiflorus*, *L. concinnus*), Espuelita (*Delphinium scaposum*), Toloache (*Datura meteloides*), Juan loco (*Nicotiana glauca*), Tabaco de coyote (*Nicotiana trigonophylla*), Hierba loca (*Astragallus* spp.), Cardo (*Argemone mexicana*), Garbancillo (*Crotalaria pumila*), Huinar and escobilla silvestre or escobilla morada (*Melochia pyramidata*) (Sáenz, 1964; Breuer *et a*l.,1982; Bruneton, 2001; Tokarnia, 2002; Haraguchi, 2003).

The huinar (*M. pyramidata*), is a perennial herb and belongs to the family Sterculiaceae, it is a plant from 1 to 1.5 meters high, very branched; it has a purple flower, and hence one of its common names is derived. The stems are of soft consistencies but very resistant; the leaves are 3-7 cm long, are thin, green, with hairs strewn and scattered; the edges are jagged and the leaf blade is light. The flowers are almost always pedicelled, arranged in frequently umbellate peaks that leave the armpits of the leaves; the calyx is lobed from 3 to 4 mm long, the petals are between violet and pink, measuring 6 to 8 mm. The fruits are capsules wider than long, and are between 5-6 mm in length, their shape is pyramidal. The huinar has a wide range of distribution; it is found up to 1200 meters high, in sites free of dense, high vegetation, such as areas for annual crops, orchards on periodic rest, poor uncultivated land, roadsides and roads (Breuer *et al*., 1982; Baudilio, 2009). In Mexico *M. pyramidata* has been found in the states of Campeche, Colima, Quintana Roo, San Luis Potosi, Sinaloa, Sonora, Tabasco, Veracruz and Yucatan (Avendaño and Flores, 1999).

The aerial parts of the plant contain Melochinine, from the group of pyridine alkaloids and produce paralysis, brachycardia, hypotension and death. It causes paralysis of the posterior train, notable photophobia, mucous ulcerations, severe constipation and death (Breuer *et al*., 1982). However, the studies concerning this toxic plant are scarce, the pathogenesis of its effects, the signs and the injuries it produces on cattle are not known. In Mexico there are no published reports of lesions associated with the consumption of M. pyramidata.

The objective was to describe three cases of bovines intoxicated by *Melochia pyramidata* in Colima State, Mexico.

MATERIAL AND METHODS

The cases were presented in September 2017 in Armería municipality, Colima (located between parallels 18° 51' and 19° 09' north latitude; meridians 103° 53' and 104 °08' west longitude; altitude between 0 and 1200 m) (INEGI, 2009). The productive unit was made up of 200 animals crossing from Cebu, in poor corporal conditions (2/5). The animals grazed on rainforest pastures with Guinea grass, without additional food supplement. In all the prairies there were plants of huinar, with evidence of consumption of its leaves and stems.

Three animals presented clinical signs of nervous origin, manifested initially by the difficulty to lift the pelvic limbs during walking and incoordination (ataxia). The march was aggravated until presenting paralysis of the later train (weary) and prostration; the animals show no loss of sense and appetite, consuming green fodder and water. The physiological constants were normal (respiratory rate 36/minute, heart rate 52/minute and temperature 38 °C). From each diseased animal, 5 ml of peripheral blood was collected in vacutainer tube with anticoagulant, for hematology tests. Between 3 and 5 days the animals prostrated without stopping to eat green fodder and water; they finally died. The corpses were sent to the Pathology Laboratory of the Faculty of Veterinary Medicine and Zootechnics of the University of Colima, for *post-mortem* study.

In the necropsy, the right cerebral hemisphere of each animal was placed in a sterile bottle and sent to the State Committee for the Promotion and Protection of Livestock of Colima, for diagnosis of rabies by direct immunofluorescence. In the revision of the digestive system, the rumen was found to be plethoric with forage from Guinea, interspersed with semi-digested leaves. In addition, samples from various organs were fixed in 10 % buffered-neutral formalin for histopathology. The fixed tissues were processed for inclusion in paraffin, cut to 5 µm in thickness and stained with Hematoxylin-Eosin (HE). Also samples of brain, cerebellum, medulla oblongata, thoraco-lumbar segment of spinal cord and sciatic nerves were stained with Luxol fast blue stain (Prophet *et al*., 1995). The plants (leaves, stems and flowers) of M. pyramidata were sent to the laboratory of toxicology of the FMVZ-UNAM to determine alkaloids, by the method of extraction, purification and fractionation of the extract for acids, basic and neutral (Repetto and Repetto, 2009).

RESULTS

The plants collected presented brown, resistant stems; its leaves measured 3 to 7 cm long, thin, green, with stellate hairs and jagged edges. The flowers were purple and pedicelled, arranged in frequently umbellate peaks that leave the armpits of the leaves; the chalice was lobed from 3 to 4 mm long; the petals were between violet and pink and measured from 6 to 8 mm, (figures 1-3).

In the hematology studies, leukocytosis was determined by neutrophilia, normocytic normochromic anemia, and blood smears were negative for the presence of *Babesia* sp and *Anaplasma* sp. At the necropsy of the 3 cattle, all the subcutaneous tissue exhibited edema (anasarca). The thoracic and abdominal cavities were occupied by varying amounts of white-translucent fluid (hydrothorax and ascites respectively), which was also visible within the pericardial sac (hydropericardium). Pericardial adipose tissue was sparse, soft and gelatin-like (serous atrophy of fat). Likewise, dilation of the right ventricle and pale areas within the myocardium were found (Figures 4-6). The degenerative and vascular changes found are consequences of the body condition and malnutrition of the animals. The rest of the organs did not present evident macroscopic lesions.

In the histopathological study, the lesions were restricted in the cerebellum, medulla oblongata, spinal cord, peripheral nerves and heart. In the brain, cerebellum and medulla oblongata, the neuropil showed multiple clear vacuoles corresponding to demyelination (spongiosis) and in them, some axons were observed intensely eosinophilic, tortuous and swollen (axonal degeneration). In the ventral portion of the spinal cord, the white matter showed areas similar to those described in cerebellum and medulla oblongata. The axons of peripheral nerves were observed tortuous, swollen (axonal degeneration) and surrounded by multiple clear vacuoles (demyelination), (Figures 7-11). The areas of demyelination described in the cerebellum, medulla oblongata, spinal cord and peripheral nerves were evident by Luxol fast blue staining (Figures 13-15). In the sections of the heart, at the level of the myocardium, there are some areas whose cardiomyocytes were retracted, eosinophilic, with loss of striations and vacuolization of the sarcoplasm (necrosis), (Figure 12). Immunofluorescence analysis for the determination of rabies virus was negative. In the toxicology studies, the presence of three different alkaloids (without determining their type) was determined in the stems, leaves and root of *M. pyramidata*.

DISCUSSION

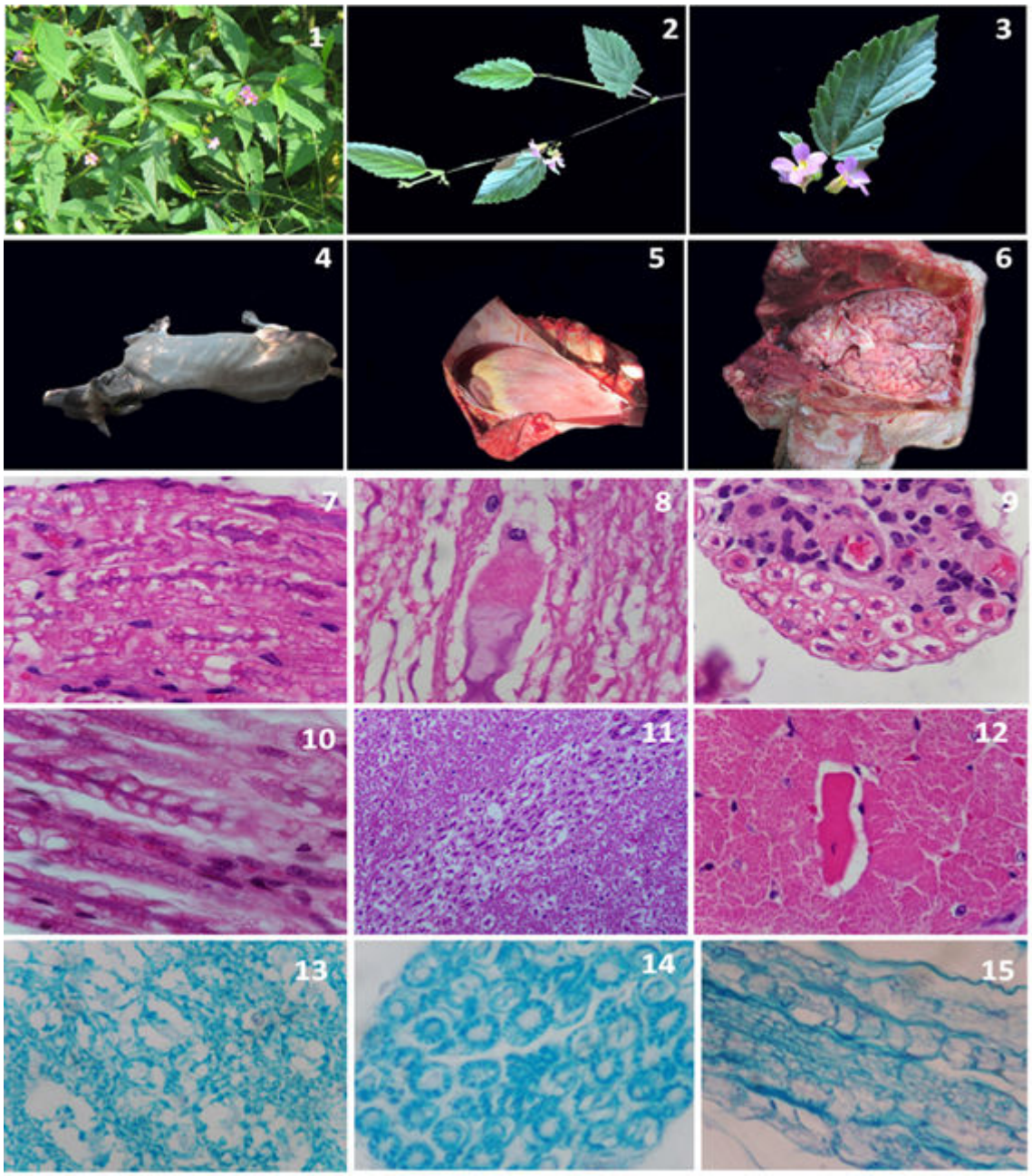
In Mexico, no documented and accurate records were found on intoxication due to cattle culling, however, in Colima state it is a frequent problem due to the loss of animals. It is not easy to obtain information about the cases of intoxication in cattle, and it is because the reports are confusing and incomplete; in addition to the difficulty to identify the toxic flora of the region, by cattlemen and veterinarians (Avendaño and Flores, 1999).

The characteristics of the plant were consistent with *M. pyramidata* and were similar to those described by other authors (Breuer *et al*., 1982, Baudilio, 2009). In this case, evidence of semi-digested runoff was found in the rumen, in addition to the revision of the paddock presence and consumption of leaves and stems was determined. This is due to the fact that in the dry season pastures are poor in forage, there is overgrazing, so the presence and availability of the plant, as well as the lack of food supplements favor the consumption of this plant (Avendaño and Flores, 1999; Bruneton, 2001; Haraguchi, 2003).

The disease of bovine cattle due to intoxication of huinar is known as "derrrengue" or "derriengue" (weary). It appears more frequently in the months of February, March and April, when grazing becomes more difficult, due to the scarcity of food and water. When grass is scarce in the pasture, due to the lack of adequate food, cattle can ingest it and become intoxicated and must be differentiated from: infectious diseases: bacterial (listeriosis, thrombotic meningoencephalitis, tetanus, botulism), viral (infectious bovine rhinotracheitis, rabies) , malignant catarrhal fever), parasitic (babesiosis); metabolic diseases (polioencephalomalacia, hypomagnesemia, metabolic imbalance Ca-P and ketosis); toxic diseases: toxic plants (*Cynodon dactylon, Phalaris, Condalia microphylla*), hepatotoxic encephalopathy, mycotoxins (*Neothyphodium lolii, Claviceps paspali, Diplodia maydis*); heavy metals and organic substances (lead poisoning, organophosphate poisoning and urea poisoning); in addition to traumatic injuries (Cantile and Youssef, 2016; Miller and Zachary, 2017).

Paralysis or weary in cattle caused by swine ingestion is a consequence of the presence of alkaloids, such as melochinine (6 - [(11R) -11-hydroxydodecyl] -3-methoxy-2-methyl-1H-pyridin- 4-one), which resembles Piericidin A (NADH dehydrogenase inhibitor). The effect of melochinine is at the cellular level, damaging the mitochondria, inhibiting the respiratory chain and the synthesis of energy. The melochinine is absorbed in the digestive system, is metabolized and distributed at the plasma level, causing paralysis of the intestine, causing tympanism. Other effects of this alkaloid are on the cardiac muscle, nerve cells and the retina; causing photophobia, blindness and hemeralopy or day blindness, as has been reported (Baudilio, 2009). Subsequently, it affects the nerves of the hind limbs, causing a degeneration of the nerve fibers, causing paralysis due to neuromuscular damage, causing a slow depolarization by reduced velocity in the Na and Ca channels (Breuer *et al*., 1982; Bruneton, 2001; Haraguchi, 2003, Cristóbal, 2007, Armién *et al*., 2009, Baudilio, 2009). No inflammatory reaction and white muscle disease were observed, so deficiency of vitamin E or selenium was ruled out. The necrosis of cardiac fibers was associated with the presence of the alkaloid on the Na and Ca channels (Armién *et al*., 2009; Baudilio, 2009).

In some reports of bovines intoxicated by huinar, no histological lesions of nervous tissue were detected, due to the incorporation of melochinine in the cell membrane, which do not



Figures 1-3. Characteristics of *M. pyramidata*. In it, it can see the plant with brown stem; green leaves, thin and jagged edges. In the same way the flowers are purple and pedicelled, and show petals of color between violet and pink. Figure 4. In this image one of the remitted animals can be seen, without external alterations. Figure 5. Observe the hydropericardium and epicardial degeneration. Figure 6. Macroscopically the brain did not show significant alterations. Figures 7-11. Photomicrographs corresponding to encephalon and one of the peripheral nerves, where the white matter shows marked vacuolization (spongiosis) and axonal degeneration. Hematoxylin-Eosin (HE)/10 and 40X. Figure 12. Photomicrograph of the heart which shows some degenerated and necrotic cardiomyocytes. HE/10X. Figures 13-15. Special stain of Luxol fast blue, in which the areas of demyelination described in encephalon and peripheral nerves are evident. 10 and 40X.

necessarily lead to detectable microscopic lesions (Haraguchi, 2003); contrary to this study, where the lesions were degeneration and demyelination of the nerves and spinal cord, perhaps due to excessive consumption of the plant.

In Colima, intoxication is common due to cattle ranching, causing their death. The report of these three cases can help farmers and veterinarians to identify the toxic flora of the region, know its effects, signs, injuries and consider it as a differential diagnosis of diseases that affect the nervous system.

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