HEPATIC ENCEPHALOPATHY INDUCED BY SEEDS AND PODS OF Senna occidentalis IN PIGS

Encefalopatía hepática inducida en cerdos por semillas y vainas de Senna occidentalis

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ABSTRACT

Senna occidentalis is a toxic plant that affects different animal species. The predominant lesion found in most of the intoxicated animals is skeletal muscle degeneration. However, in horses and humans, this poisoning is primarily characterized by hepatic encephalopathy (HE). The aim of this paper was to determine whether, in addition to this myodegeneration, the seeds and pods of S. occidentalis induce toxic EH in pigs. Ten pigs were divided into two groups (of five animals each), one of which were fed with a ration containing 20 % of S. occidentalis pods and seeds, and the other with a commercial ration (control) for 14 days. Poisoned animals had a sudden onset of symptoms, characterized by incoordination, ataxia, disorientation and head pressing, depression and lateral recumbency. Aspartate aminotransferase and Creatine phosphokinase serum activities increased along with an increase in serum bilirubin in intoxicated animals with S. occidentalis. Histopathological studies of the poisoned pigs showed hepatocellular swelling and centrilobular necrosis in the liver, vacuolization of the white matter and Alzheimer type II astrocytes in the cerebral cortex of the brain. Electron microscopy revealed mitochondrial lesions in liver. These results showed that in this study, the muscle injury previously reported was not observed, most probably because the toxic EH reproduced in the evaluated animals were produced before skeletal muscle degeneration occurred. On the other hand, the animals of the present study developed clinical signs and histological lesions that were similar to those observed in cases of accidental poisoning. Besides, further studies are needed to identify the specific toxin responsible for acute liver failure, observed in the animals of this study.

Key words: Hepatic encephalopathy; pig; poisoning; toxic plant.

RESUMEN

Senna occidentalis es una planta tóxica que afecta a diferentes especies de animales. La lesión hallada en la mayoría de los animales intoxicados es la degeneración muscular. Sin embargo, la encefalopatía hepática (EH) es observada en equinos y en humanos. El objetivo de este trabajo fue determinar si, además de la miodegeneración ya reportada, semillas y vainas de S. occidentalis inducen una EH tóxica en cerdos. Diez animales se dividieron en dos grupos (de cinco animales cada uno), uno de los cuales se alimentó de una ración que contenía el 20% de vainas y semillas de S. occidentalis, y los animales controles recibieron ración comercial durante 14 días. Los animados intoxicados presentaron síntomas de aparición brusca, caracterizados por incoordinación, ataxia, desorientación y presión de la cabeza contra objetos duros, depresión y recumbencia lateral. La aspartato aminotransferasa y creatinquinasa aumentaron junto con la Bilirrubina Total en los animales intoxicados. Los estudios histopatológicos de los cerdos alimentados con S. occidentalis mostraron tumefacción hepatocelular y necrosis centrolobular en el hígado; mientras que el encéfalo presentó vacuolización de la sustancia blanca y astrocitos Alzheimer tipo II en la corteza cerebral. La microscopía electrónica reveló lesiones mitocondriales en el hígado. Estos resultados muestran que en el presente estudio, la lesión muscular ya reportada no se observó, seguramente debido a que la EH tóxica reproducida en los animales evaluados se produjo antes que la degeneración muscular. Además, futuros estudios son necesarios para identificar el tóxico responsable de la falla hepática aguda, observada en los animales de este estudio.

Palabras clave: Encefalopatía hepática; cerdos; intoxicación; planta tóxica.
INTRODUCTION

Several species of the genus Senna are toxic, but *S. occidentalis* and *S. obtusifolia* are considered to be more toxic than others [14, 34]. These legume plants can be found in open pastures and in fields cultivated with cereals such as maize (*Zea mays*), soybean (*Glycine max*), sorghum (*Sorghum spp.*) and others. Thus, during harvest, it is almost impossible to prevent these plants from mixing up with the cultivated crops [20]. The seed of these plants is considered the most toxic part [22].

The literature extensively reports both experimental and spontaneous cases of poisoning by *S. occidentalis* in many tropical and subtropical regions around the world, including Africa [11], the USA [13, 15], Asia [27], Australia [29], and many areas of Central [12] and South America [24, 25]. In northern Argentina, *S. occidentalis* is known with the common name of “cafeillo”.

The predominant lesion caused by this toxic plant in cattle (*Bos taurus* y *Bos indicus*), pig (*Sus scrofa domesticus*) and wild boars (*Sus scrofa*) is degenerative myopathy of striated muscles [6, 23, 30]. However, in horses (*Equus caballus*), other lesions characterized by hepatic encephalopathy, a severe neurological disorder in the absence of normal hepatic function, have also been observed in spontaneous episodes of intoxication with *S. occidentalis* seeds [25]. There are also some reports of toxicity in humans, with toxic injuries to the liver, muscle and brain [33, 34]. Although clinical evidence of brain disease is present, this aspect has not been extensively studied in other species such as pigs. Thus, the aim of the present study was to determine whether, in addition to myodegeneration previously reported, seeds and pods of *S. occidentalis* induce a toxic HE in pigs.

MATERIALS AND METHODS

Plant material

Ripe *S. occidentalis* seeds and pods were collected from Corrientes City, Corrientes Province, Argentina, in March 2015. A voucher herbarium specimen was deposited at the Instituto de Botánica del Nordeste (IBONE), Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina, under the number CTES-540. After harvesting, seeds and pods were immediately triturated and incorporated to the ration provided to pigs. To rule out the possible contamination of rations, an ELISA was performed to determine mycotoxins by using the AgraQuant® total Aflatoxin Test Kit.

Animals and experimental design

Ten 6-week-old pigs (mean weight: 21.35±1.87 kilograms (kg)) were supplied by the Facultad de Ciencias Veterinarias – UNNE and separately housed. All animal procedures were approved by the Ethics at Biosafety Committee of the Facultad de Ciencias Veterinarias - UNNE (0068-2016).

The animals were randomly divided into two groups: treated animals received a ration containing 20% of *S. occidentalis* pods and seeds, and the control group received a commercial ration. The intake of rations was approximately 3% of their body weight (BW) per day (d). In all groups, water was administered *ad libitum*.

Feed intake of each pig was recorded daily and pigs were weekly weighed (digital balance Kretz IDP 5640 ECO, Santa Fe, Argentina). Blood samples were collected, to perform a hemogram and to determine serum enzyme activities and serum total bilirubin. Immediately, after they were anesthetized by an intramuscular injection of ketamine hydrochloride (5 miligram (mg)/kg) and xylazine hydrochloride (0.2 mg/kg), animals were euthanized on d 14.

Histopathology

For routine histological analysis, multiple tissues (kidney, skeletal and cardiac muscles, liver, whole brain) were collected from euthanized animals, fixed in phosphate-buffered formaldehyde and embedded in paraffin. Samples were cut into 5 micra (µm) serial sections, stained with hematoxylin and eosin (H–E) and examined with a Primo Star Zeiss microscope, Germany. Images were taken with an Axiocam ERc 5s Zeiss digital camera, Germany.

Transmission electron microscopy (TEM)

Immediately after sacrifice, samples of liver were pre-fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2-7.4, and post-fixed in osmium tetroxide and embedded in epoxy resin. Semi-thin sections (1 µm thick) were stained with 1% toluidine blue in 1% borax. Ultra-thin sections (60–80 nm) of selected areas were stained with 2% uranyl acetate and lead citrate and examined with a JEOL EM 1200EX II, Tokyo, Japan TEM.

Statistical analysis

Values were expressed as means ± standard deviation (SD). Data were statistically analyzed by ANOVA followed by Tukey test (InfoStat) [9]. The level of significance was set at P < 0.05.

RESULTS AND DISCUSSION

Diagnosis of intoxication by *S. occidentalis* is based on clinical signs and pathological findings [2, 3, 30, 32]. In the present study, the BW of treated animals was significantly lower than those of control animals (P < 0.05). *S. occidentalis* consumption by pigs, maintained their BW for the first week. After, they had a net loss BW of 0.60 ±0.08 kg/d. The mean BW of this group at 14 d was 15.73±1.75 kg. In contrast, control pigs had a BW gain of 0.44±0.06 kg/d and a final BW of 27.70±1.82 kg. No mycotoxins (aflatoxins A and B) were detected in the ration samples, as evaluated by ELISA. Besides, the pigs consuming *S. occidentalis* presented a sudden onset of symptoms, characterized by incoordination, ataxia, disorientation and head pressing. These
animals were reluctant to move, and clinical signs progressed to severe depression and recumbency. They were thus sacrificed on d 14. These symptoms are in agreement with description of experimental poisoning in many animal species with this plant [1, 7, 10, 16, 17, 19, 28, 31].

As shown in TABLE I, *S. occidentalis* poisoning in pigs caused significant elevations of serum activities of aspartate aminotransferase (AST) and creatine phosphokinase (CK) enzymes, as well as an increase of serum bilirubin compared to the control group (P < 0.05). Hematological values (hematocrit, hemoglobin concentration and red blood cells) remained similar to the control group (data not shown). These changes had not been previously described in pigs. Unfortunately, ammonia concentration was not investigated in the present study. However, it is known that ammonia reaches the brain and it is detoxified in astrocytes by means of enzyme glutamine synthetase [4]. It could be speculated an increasement in brain ammonia because in the evaluated animals it has been observed astrocyte swelling and brain oedema in the pigs.

However, several researchers have reported increase in serum AST and CPK activities in other *S. occidentalis* poisoned animal species [21, 25, 30]. Detection of serum enzymes tests may be of early diagnostic value in intoxication by *S. occidentalis*.

At necropsy, animals that received *S. occidentalis* evidenced cerebral oedema, which caused compression of the circonvolutions, mainly at cerebellar level; additionally, the liver of these pigs had pale areas and light brown, compared to control animals (FIG. 1 a-b).

The main histological lesions were observed in liver and Central Nervous System (CNS) of pigs treated with *S. occidentalis*. The liver had a panacinar acute hepatocellular swelling and multiple small foci (3 to 40 cells) of coagulative necrosis of hepatocyte located in zone 3 and periacinar zone. Furthermore, intracellular hyaline in swollen hepatocytes and acidophilic bodies (hepatocyte apoptosis) were also found (FIG. 2. a). There were no pathological changes in the control animal (FIG. 2. b). Cerebral cortical lesions were found in these pigs. Hypertrophied astrocytes with scant cytoplasm, vesicular nucleus, marginated chromatin were frequently observed in pairs (Alzheimer type II astrocytes); and also severe vacuolization in the white matter of the brain, cerebellum and brain stem (spongiosis) was found (FIG. 2. c-d). The histopathological study of pigs from control animals revealed no lesions in the examined organ (FIG. 2. e).

Differing from the previously described poisoning of *S. occidentalis* toxicity in pigs [7, 23, 28], histological changes in the present study were restricted to the liver and brain, which was reproduced with 20% of *S. occidentalis* seeds and pods. Nonetheless, muscle lesions of the current pigs were absent (FIG. 2. f-g). According to Vashishtha *et al.* [34], Senna induces acute liver degeneration and death before myodegeneration has time to develop.

The pathology in the present study have been characterized by hepatic necrosis and hepatocellular swelling and spongiosis and Alzheimer type II astrocytes in the cerebrum. These are lesions related to encephalopathy and are typical histological findings associated to hyperammonaemia in different species of animals, consequent to either hepatic disease [8]. In according to Panigrahi *et al.*, [26], who detected different anthraquinone aglycones in the serum and urine samples of humans with hepatomyoencephalopathy (HME), and in rats (*Rattus norvegicus var. albinus*) exposed to *S. occidentalis*; these authors revealed that rhein is the most cytotoxicity of the anthraquinones in rats primary hepatocytes.

Despite of not characterizing the toxics in *S. occidentalis*, it was speculated that the hepatic damage followed by brain damage is associated to a direct insult to the liver due to toxins present in *S. occidentalis*, and not to a direct damage to the brain, as reported by Barbosa-Ferreira *et al* [1] and Oliveira-Filho *et al.* [25].

Transmission electron microscopy of the liver from intoxicated pigs fed a ration with 20% of *Senna occidentalis*. a) Light-brown color of the hepatic surface and diffuse paleness. b) Gross photograph of a control liver.

### TABLE I

<table>
<thead>
<tr>
<th>Group (n =5)</th>
<th>TB mg/dL</th>
<th>CPK (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>4.15±0.4*</td>
<td>6762.33±1600.05*</td>
<td>1064.50±170.211*</td>
</tr>
<tr>
<td>Control</td>
<td>0.14±0.07</td>
<td>482±52.12</td>
<td>37.23±1.31</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD. P < 0.05 versus control group. TB: Total bilirubin, CPK: Creatine Phosphokinase, AST: Aspartate aminotransferase.*
Poisoning in pigs induced by toxic plant / Soledad Chileski, G. y col.

Pigs revealed dilated mitochondria and cristolysis (FIG. 3). The present study confirmed the previous findings from other animal species, using TEM [5, 13, 31], it was detected mitochondrial disturbances, mainly in hepatic cells of the current pigs. Some authors reported that mitochondria is the target organelle of the toxic active principles of *S. occidentalis* [18]. However, the miotoxic compounds of *S. occidentalis* and intrinsic mechanism by which these toxics causes mitochondrial impairment is still unknown, [32], though a toxic compound named dianthrone has been identified in seeds [16]. This is an anthraquinone-derived compound associated with mitochondrial myopathy. Additionally, according to Barbosa-Ferreira *et al.*[1] and Oliveira-Filho *et al.* [25], dianthrone could cross the blood-brain barrier and cause damage in the CNS of rats and horses, respectively. On the other hand, recently studies indicate that other anthraquinone aglycones, mainly rhein, are responsible for producing HME in humans and rats previously mentioned [26]. Thus, future studies should be done to investigate about the toxics compound responsible for development of this syndrome observed in the evaluated animals.

**CONCLUSIONS**

It was concluded that 20% *S. occidentalis* poisoning in pigs causes, predominantly, a toxic hepatic encephalopathy with absence of muscle lesions. All animals had developed clinical signs and histological lesions that were similar to those observed in cases of accidental poisoning. Besides, further studies are needed to identify the specific toxin responsible for acute liver failure, observed in the animals of this study.

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