Toxicon 216 (2022) 57–64

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Experimental intoxication of Brahman (*Bos indicus*) heifers with *Enterolobium cyclocarpum* fruits

María C. Lozano [a,*], Leonardo Roa [b], Carlos A. Moreno [c], Noel Verján-García [d], Benjamín Doncel [e]

[a] Facultad de Ciencias, Departamento de Farmacia, Universidad Nacional de Colombia, Colombia
[b] Laboratorio de Patología Veterinaria- Facultad de Ciencias Agropecuarias, Universidad de Ciencias Aplicadas y Ambientales (U.D.C.A), Bogotá, Cundinamarca, Colombia
[c] Laboratorio Clínico, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Bogotá, Colombia
[d] Laboratory of Pharmacology and Toxicology, James Graham Brown Cancer Center, University of Louisville School of Medicine, USA
[e] Laboratorio de Patología Veterinaria, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Bogotá, Colombia

ARTICLE INFO

Handling Editor: Ray Norton

Keywords:
Cattle
Colombia
Enterolobium cyclocarpum
Toxic plants

ABSTRACT

Enterolobium cyclocarpum is a poisonous plant distributed throughout the Americas. The *E. cyclocarpum* fruits have high toxic potential for cattle in Colombia and the clinical signs and pathological lesions are ill-defined. To begin address this issue, twelve Brahman heifers were administered *E. cyclocarpum* fruits and the evolution of clinical signs were recorded. Blood was collected to establish biochemical and hematological parameters. Animals were euthanized between 4 and 15 days after the initial dose was given, and tissue samples were routinely processed and stained by Hematoxylin-Eosin. The severity of clinical signs and tissue lesions were correlated with the dose of *E. cyclocarpum* fruits. Clinical signs included fever, tachypnea, sialorrhea, jaundice, tympanism, and diarrhea. Skin lesions were consistent with photosensitization. Hematological and biochemical tests showed increased hematocrit, neutropenia, increased serum fibrinogen, elevated hepatic enzymes and azotemia. Histology revealed panlobular cytoplasmic vacuolization and extensive foci of necrosis in the liver. The skin, forestomach, abomasum and intestine revealed microcirculatory, inflammatory and ulcerative changes. Protein casts and tubular epithelium vacuolization were found in kidney. Depending on the toxicosis intensity, it is concluded that *E. cyclocarpum* fruits may cause two clinical and pathological forms of poisoning in Brahman heifers. First, a severe intoxication at repeated exposition with high (20 g/kg/d) or low (10 g/kg/d) dose that affected the digestive and tegumentary systems and the kidney. Second, a mild to moderate form with a single low dose (10 g/kg/d) that affected in lower grades the same systems/organs.

Author contribution

María C. Lozano: Project administration, funding acquisition, writing - review and editing. **Leonardo Roa:** Investigation, methodology, animal manipulation, histopathological analysis, writing - original draft. **Carlos A. Moreno:** Investigation, methodology, clinical and clinical pathology evaluation. **Noel Verján:** Writing - review & editing, **Benjamín Doncel:** Investigation, methodology, funding acquisition, histopathological analysis.

1. Introduction

The predominant cattle production system in the Eastern Plains of Colombia is an extensive type of livestock production carried out on topographies such as the flat “altillanura”, “serranía” and flooded savannahs. This type of livestock production is characterized by low quality of forages, low productivity and frequent cases of plant intoxication. In Colombia, there are no accurate and up-to-date studies on the economic losses of cattle due to plant poisoning. The toxic effect of leguminous plants from the *Fabaceae* family on cattle breeding in Colombia has not been clearly documented and the scarce information corresponds mainly to empirical data. Torres et al. (1983), reported economic losses for cattle raising, close to COP 2000 million per year, represented by sudden deaths, reproductive disorders, discards, chronic pain, palliative treatments, control measures and prophylaxis that were like those reported in Brazil (Riet Correa and Medeiros, 2001). Assuming a 0.5% cattle mortality attributed to poisonous plants (Mello et al., 2010), in Colombia Eastern Plains, particularly in Meta and Casanare

* Corresponding author. Cr. 30 N°. 45-03, Departamento de Farmacia (Ed. 450), Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, Colombia.
E-mail address: mclozanoa@unal.edu.co (M.C. Lozano).

https://doi.org/10.1016/j.toxicon.2022.06.015
Received 28 February 2022; Received in revised form 15 June 2022; Accepted 27 June 2022
Available online 1 July 2022

departments, approximately 21,270 cattle heads die per year due to plant intoxication (ICA Censo Pecuario Nacional, 2021). In Brazil, it was estimated that between 800,000 and 1,120,000 cattle heads die annually from plant poisoning (Tokarnia et al., 2002).

Enterolobium cyclocarpum is a leguminous plant that is recognized by its extreme toxicity in cattle from the Colombian Eastern Plains and Venezuelan plains (Lozano et al., 2011; Negrón et al., 1993). In Brazil, field outbreak intoxications or experimental intoxication with other *Fabaceae, E. contortisiliquum* has been documented and clinical signs such as jaundice, anorexia, ruminal atony, photosensitization, diarrhea and prostration have been described (Costa et al., 2009; Mingatto et al., 2008), as well as abortions and death in bovines (Grecco et al., 2002; Mendonca et al., 2009; Mello et al., 2010; Mingatto et al., 2008; Sowemimo et al., 2015). Additionally, ruminal acidosis was described in sheep (Pupin et al., 2017). In Colombian Eastern Plains, the main clinical signs associated with natural *E. cyclocarpum* intoxication are photosensitization, anorexia, prostration and death (Lozano et al., 2011). Liver enzymes such as AST and GGT were elevated in cattle and sheep intoxicated with *E. contortisiliquum* (Grecco et al., 2002; Olinda et al., 2015; Pupin et al., 2017). These biochemical parameters have not been assessed in cases of *E. cyclocarpum* intoxication in cattle from Colombia.

The aim of this study was to evaluate experimentally the toxic potential of *E. cyclocarpum* fruits (pods) on cattle and to describe the clinical and pathological findings under the agro-ecological conditions of the Eastern Plains of Colombia.

2. Materials and methods

All procedures were approved by the Bioethics Committee of the Faculty of Veterinary Medicine and Zootechnics of the National University of Colombia.

2.1. Geographic location

The experiment was carried out at the Hato Casona, located at the Brisas del Ovejas village of the Mapiripán Municipality, Meta. The cow farm has the following coordinates: Longitud: 2°50′49.3 "N, Latitude: 72°23′01.3″ W, Altitude: 214 m. a.s.l and T ° 26.5 °C, and relative humidity between 75 and 80% in winter period.

2.2. Collection of plant material and experimental animals

Fresh fruit pods of *E. cyclocarpum* identified in the Colombian National Herbarium with the voucher COL 511757, were collected between April and May (fructification period) from cattle farms where cases of plant intoxication were previously registered (Lozano et al., 2011). Twelve Brahman heifers with an average age of 1 year ±2 months and 150 ± 10 kg body weight were used in the experiment. Animals were grazing in natural savannah with *Trachypogom vestitus*, supplemented with 8% phosphorus mineralized salt, and 1 kg/day of commercial feed per animal and water *ad libitum* for 15 days before the experiment.

2.3. Experimental groups

The animals were randomly distributed into four experimental groups of three individuals each. Control group 1 (T1) had no access to the *E. cyclocarpum* fruits (pods). Heifers in T2 were given a single dose (10 g/kg), whereas heifers in T3 were given 10 g/kg daily and heifers in T4 were given 20 g/kg daily for a planned 15 days experimental period. The rations were supplied in the morning for voluntary intake before getting access to the traditional forage and commercial feed. Table 1 shows the experimental design used and the clinical evolution of Brahman heifers exposed to *E. cyclocarpum* fruits in the Colombian Eastern Plains.

2.4. Clinical examination

All animals were subjected to a clinical examination before the experiment begun (time zero, t0). The results of which were recorded in their respective clinical records according to Terra and Reynolds (2021). The animals were observed periodically throughout the period of plant exposure and when behavioral alterations like depression, anorexia, prolonged decubitus, diarrhea or tympanism were detected, the animals were again clinically evaluated. Those clinical signs were recorded at time 1 (t1), and the last clinical examination was conducted at time 2 (t2), when either, experiment period had finished (15 days), or the clinicians by consensus established animals were suffering, bearing the welfare considerations previously accorded between members of the research group and the bioethics committee (Olfert et al., 1993). After this, euthanasia and necropsy were performed. Blood samples were collected at each time of clinical evaluation, and they were sent to the Faculty of Veterinary Medicine and Zootechnics of the National University of Colombia, to be analyzed for various biochemical parameters.

2.5. Clinical pathology

Blood samples were collected in vacutainer® tubes with EDTA anticoagulant for complete blood count and without anticoagulant for serum collection and aspartate amino transferase (AST), gamma glutamyl transferase (GGT), urea and creatinine (CREA) measurements. Hematological analyzes, consisting in erythrocytic and leukocytic evaluation including fibrinogen content, were performed with a Nihon Kohden® automated equipment and biochemical tests were performed on a Vitros DT60II 14® (Johnson and Johnson) equipment. Leukocyte differential counting was made with optical microscopy on a Wright-stained blood film on total 200 cells.

2.6. Pathology

Euthanasia was conducted by administration of sodium pentobarbital at a dose of 60 mg/kg intravenously (Shaw and Reilly, 2001). Immediately, a standard necropsy was performed, and macroscopic

Table 1
Doses and severity of clinical signs in Brahman heifers exposed to *E. cyclocarpum*.

Treatment (T)	Doses	N°. of doses	Total dose g/kg	Number of days of experiment	Beginning of clinical signs (day after initial exposure)	Severity of clinical signs
T1 (Control)	NE	NE	NE	NE	NE	No clinical signs
T2	10 g/kg/d SID	Single dose	10	15	2	Transient clinical signs
T3	10 g/kg/d SID	6	60	8	2	Severe clinical signs (euthanized at day 8)
T4	20 g/kg/d SID	3	60	4	1	Severe clinical signs (euthanized at day 4)

NE: Not exposed to *E. cyclocarpum* pods.

findings recorded. Tissue samples (forestomaches, abomasum, gut, liver, kidney, skin, encephalon) were preserved in 3.7% buffered formaldehyde and processed by the routine Hematoxylin-Eosin (HE) staining (Luna, 1968). Histopathological analysis included microcirculatory changes such as inflammatory and morphological alterations. A qualitative score of four levels of severity was established: 0 = normal, when there was no tissue change; 1 = mild, when there was a perceptible lesion without evident architectural alteration of the tissue; 2 = moderate, when the tissue showed an evident lesion of multifocal distribution, with incipient changes of the architecture; and 3 = severe, when there was an evident and extensive lesion with architectural alteration of the tissue. Scores were averaged according to treatment; decimals were approximated to whole number.

2.7. Statistical analysis

All qualitative and quantitative data was tabulated in Excel (2010)®. A descriptive analysis was performed on the clinical findings. In addition, a completely randomized design was used for quantitative variables such as blood count, liver function enzyme (AST, GGT) and renal metabolites (urea and CREA).

An analysis of variance was conducted to compare quantitative variables between treatments at different times of the toxic process (t0, t1 and t2), with the Tukey test (HSD). Statistically significant differences were considered when $p < 0.05$. The programming language R (R core team, 2017) was used for time-series analysis. The pathology analyses included descriptive statistics and the independence test (chi^2), in order to establish associations between the treatments and the response variable. Significant differences were considered when $p < 0.05$.

3. Results

3.1. Clinical examination

Cattle exposed to single dose (10 g/kg – T2) of *E. cyclocarpum* pods showed on second day, transient signs particularly moderate foamy tympanism followed by apathy, depression and decrease on food consumption. Heifers belonging to T3 received pods (10 g/kg) for 6 days, all animals on second day showed bloat, diarrhea, fever, and depression; on day 5 they presented erythema, scales, and ulceration on sun exposed

Table 2
Main hematological and blood chemistry parameters in Brahman heifers exposed to *E. cyclocarpum* fruits.

Sampling time	Parameter	Reference values	Treatments			
			T1	T2	T3	T4
Time 0 Before the experiment	Hematocrit %	28–34	33 (30–36)	33 (30–36)	34 (30–38)	33 (28–35)
	Fibrinogen mg/dL	200–700	300 (200–400)	400 (200–500)	500 (400–600)	450 (400–500)
	Leucocytes 10^3/μL	6.5–13.0	11.7 (10.0–13.0)	8.0 (7.8–8.2)	11.4 (9.0–13.8)	11.4 (9.2–13.6)
	Neutrophils %	21.3–41.5	33 (31–35)	33 (30–35)	33 (32–34)	34 (32–35)
	Neutrophils 10^3/μL	1.8–5.4	3.9 (3.6–4.1)	2.6 (2.4–2.8)	3.8 (3.7–4.0)	3.9 (3.7–4.0)
	Lymphocytes %	49.6–71.4	62 (55–70)	61 (50–60)	65 (55–70)	63 (60–67)
	Lymphocytes 10^3/μL	4.2–9.3	7.3 (6.4–8.2)	4.9 (4.0–4.8)	7.4 (6.4–8.2)	7.2 (6.8–7.6)
	Urea mg/dL	32.4–64.8	58.0 (50.3–64.2)	45.8 (40.4–55.8)	30.8 (25.5–40.3)	32.9 (30.1–40.7)
	CREA mg/dL	1–2	0.97 (0.8–1.2)	0.92 (0.7–1.2)	0.95 (0.9–1.1)	0.97 (0.9–1.4)
	GGT IU/L	20.5–32.0	30.3 (27.5–32.0)	28.6 (25.3–30.1)	22.1 (21.6–23.2)	26.8 (24.2–28.2)
	AST IU/l	60.3–72.3	72.3 (65.2–76.1)	62.6 (60.4–66.8)	60.9 (58.6–63.4)	60.3 (56.9–62.0)
Time 1 1 to 2 days after experiment beginning	Hematocrit %	28–34	36 (34–39)	34 (32–35)	**41** (35–43)	**52*** (48–53)
	Fibrinogen mg/dL	200–700	600 (550–700)	**1100*** (900–1300)	**850** (800–1000)	**1300*** (1200–1400)
	Leucocytes 10^3/μL	6.5–13.0	12.4 (9.0–13.2)	12.7 (11.5–13.1)	7.5 (7.2–8.0)	10.8 (10.0–12.3)
	Neutrophils %	21.3–41.5	33 (30–37)	38 (35–40)	28 (26–33)	**10*** (7–14)
	Neutrophils 10^3/μL	1.8–5.4	4.1 (3.7–4.6)	4.8 (4.4–5.1)	2.1 (2.0–2.5)	**1.1*** (0.8–1.5)
	Lymphocytes %	49.6–71.4	65 (60–73)	61 (56–65)	71 (66–75)	88* (86–90)
	Lymphocytes 10^3/μL	4.2–9.3	8.1 (7.4–9.1)	7.7 (7.1–8.3)	5.3 (5.0–5.6)	9.5 (9.3–9.7)
	Urea mg/dL	32.4–64.8	27.2 (26.2–32.4)	62.1 (58.0–65.7)	**98.4*** (94.3–110.2)	**301.7*** (280.5–320.1)
	CREA mg/dL	1–2	1.0 (0.6–1.3)	1.3 (1.0–1.8)	1.9* (1.8–2.5)	**2.2*** (2.1–2.5)
	GGT IU/L	20.5–32.0	25.4 (24.8–28.5)	**36.2** (34.5–40.1)	**42.6*** (40.5–45.1)	31.2 (30.5–34.6)
	AST IU/l	60.3–72.3	77.3 (60–80)	**148.6*** (120–160)	**159.3*** (150–175)	**96.0** (75.3–116.8)
Time 2 4–15 days after experiment beginning	Hematocrit	28–34	34 (33–38)	37 (35–40)	44 (37–48)	50* (49–53)
	Fibrinogen mg/dL	200–700	500 (400–600)	**850** (500–1000)	**800*** (750–1000)	**1000*** (900–1300)
	Leucocytes 10^3/μL	6.5–13.0	12.0 (8.2–13.5)	16.0* (12.0–18.1)	15.3* (13.2–17.4)	13.2 (12.6–15.7)
	Neutrophils %	21.3–41.5	35 (30–38)	28 (26–31)	25 (22–26)	**15*** (11–17)
	Neutrophils 10^3/μL	1.8–5.4	4.2 (3.6–4.6)	4.5 (4.2–4.7)	3.8 (3.4–4.0)	**1.9*** (1.5–2.2)
	Lymphocytes %	49.6–71.4	63 (59–66)	70 (66–78)	71 (68–77)	84* (82–88)
	Lymphocytes 10^3/μL	4.2–9.3	7.6 (7.1–7.9)	11.2* (10.5–12.5)	6.0 (5.8–6.5)	11.1* (10.8–11.6)
	Urea mg/dL	32.4–64.8	52.8 (49.3–57.2)	**102.7** (75.1–135.3)	**124.1*** (119–130)	**288.9*** (200–302)
	CREA mg/dL	1–2	0.9 (0.8–1.4)	1.2 (1.0–1.6)	1.9* (1.7–2.3)	1.6* (1.5–1.9)
	GGT IU/L	20.5–32.0	22.0 (18.0–25.2)	26.3 (24.2–25.7)	**45.2*** (35.4–60.2)	**35.1*** (32.8–41.4)
	AST IU/l	60.3–72.3	72.0 (66.0–80.5)	61.6 (58.6–66.3)	**228*** (200.3–260.1)	**232*** (210–250)

Reference values from Clinic Laboratory, Faculty of Veterinary Medicine and Zootechnics of the National University of Colombia. Upper value represents the media; range of measures are indicated in brackets; n = 3. Bold font indicates relevant changes. * Statically significant differences respect control at same time ($p < 0.05$).

skin, besides jaundice; on seventh day a bovine was found on lateral decubitus and the others were atonic, diarrheic, severe depressed and had muscle tremors; cattle were euthanized on eighth day. Ruminants from T4 received daily *E. cyclocarpum* pods (20 g/kg) on four occasions, on first day they showed fever (up to 40.8 °C), tachypnea (45–50 rpm), ruminal bloat and diarrhea; on third day they were icteric, had same lesions on skin described for T3 and showed prostration, muscle tremors and opisthotonos. T4 animals were euthanized on day 6.

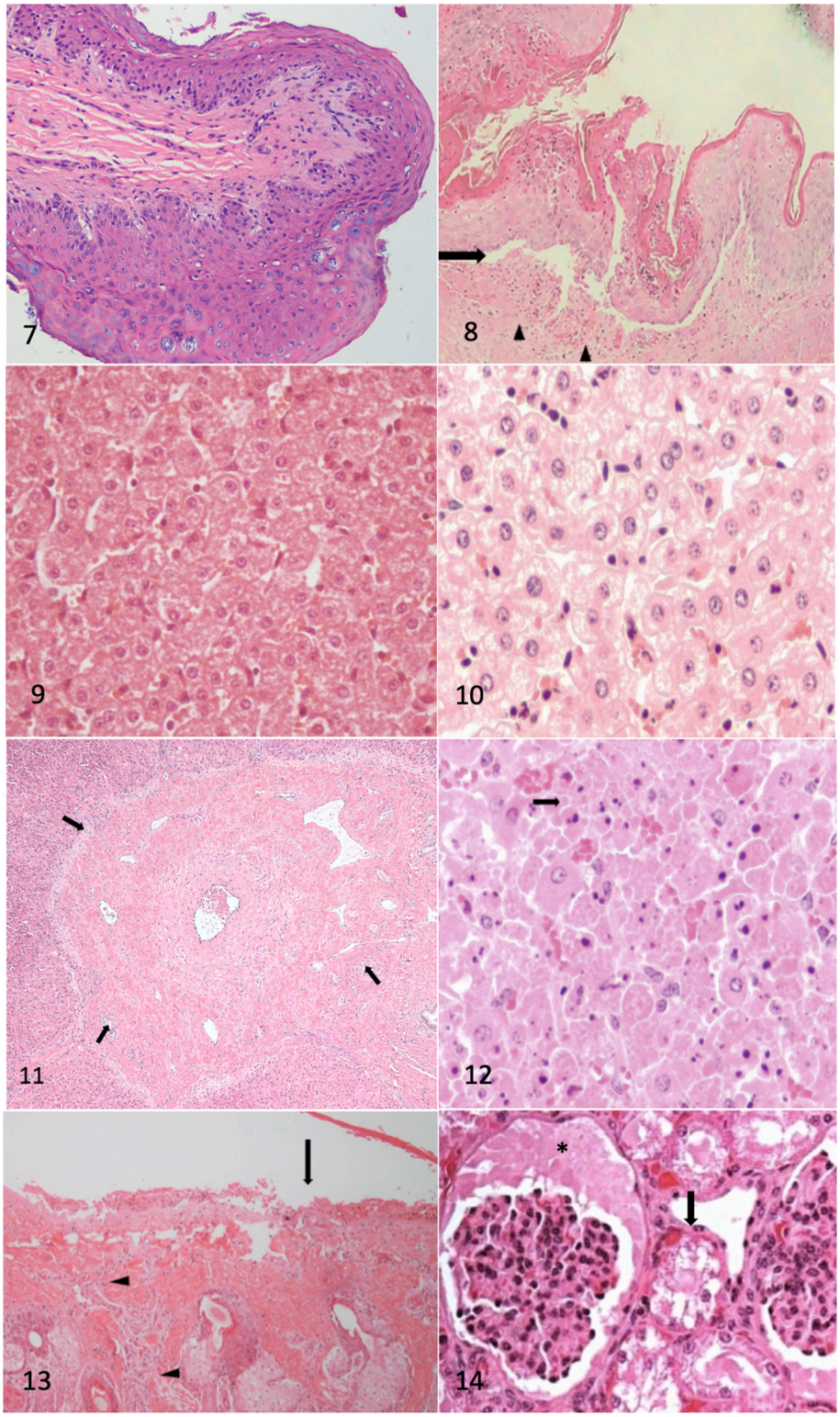
3.2. Clinical pathology

Hemogram and serum chemistry values in heifers treated with *E. cyclocarpum* fruits are present in Table 2. Hematocrit increased in T3 and T4 groups and values in T4 showed significant differences ($p < 0.05$) when compared to control animals. Relative and absolute neutrophil counts decreased severely in T4 group, with significant differences regarding control. All animals exposed to *E. cyclocarpum* fruits showed hyperfibrinogenemia with values between 800 and 1300 mg/dL and significant differences regarding control, depending on measure time.

Figs. 1–6. Gross lesions in Brahman heifers exposed to *E. cyclocarpum* pods in Colombian Eastern plains. **Fig. 1:** Erythema, ear skin, bovine T4, day 2 post exposure. **Fig. 2:** Hyperkeratosis, chest skin, bovine T3, day 7 post exposure. There is moderate ulceration. **Fig. 3:** Epithelial erosion, rumen, bovine T4, day 2 post exposure. Locally extensive epithelial erosion (arrow) in the tunica mucosa. **Fig. 4:** Mucosal congestion, reticulum, bovine T3, day 7 post exposure. Severe generalized congestion (arrow) in the tunica mucosa. **Fig. 5:** Mucosal erosion, abomasum, bovine T2. Extensive mucosal erosion (arrowhead) and moderate congestion. **Fig. 6:** Rounding liver, bovine T3. Mild rounding and moderate dilation of gallbladder.

Urea values increased and were significant different in T3 and T4 groups compared to control animals. In animals exposed to *E. cyclocarpum*, the values of urea varied between 62.1 and 301.7 mg/dL on t1 and between 102.7 and 288.9 mg/dL on t2. Serum CREA showed increases in T3 and T4 but without physiological relevance, except for T4 on t1 (2.2 mg/dL). GGT increased in cattle exposed to pods, however for T2, enzyme activity returned to normality at the end of experiment; difference respect control was statistically significant for T3 and T4 at t2.

The hepatocyte permeability marker AST enzyme increased at the beginning of clinical signs in T2, T3 and T4 groups (148.6, 159.3, 96 IU/L, respectively) and for the two former they were statistically significant different from those in control group (77.3 IU/L). At the end of the experiment (t2) the values of AST increased up to 228–232 IU/L in T3 and T4 group, respectively, and were statistically significant different to T2 and control animals.



Figs. 7–14. Microscopic lesions in Brahman heifers exposed to *E. cyclocarpum* pods in Colombian Eastern plains. Hematoxylin and eosin stain (H-E). **Fig. 7:** Rumen of control animal T1, intact mucosal surface 100X. **Fig. 8:** Epithelial detachment, rumen, bovine T3. Detachment (arrow), congestion and mixed inflammatory infiltrate (arrow heads) 100X. **Fig. 9:** Liver of control animal T1 400X. **Fig. 10:** Liver of bovine T3. Hepatocyte vacuolization. Cellular swelling 400X. **Fig. 11:** Liver of bovine T3. Portal fibrosis (arrows) 40X. **Fig. 12:** Liver of bovine T4. Multiple foci of cell death (necrosis), cellular detritus (arrows) 400X. **Fig. 13:** Extensive ulceration, skin, bovine T3. Loss of the epidermis (arrow) and mixed perivascular inflammatory infiltrate (arrow heads) 100X. **Fig. 14:** Kidney of bovine T3. Protein cylinders in Bowman space (asterisk), vacuolar changes in the cytoplasm of cortical renal tubules (arrow) 400X.

3.3. Pathology

Macroscopic findings in T3 and T4 animals included jaundice, photosensitization, and moderate to severe tympanism. Dermatological lesions were erythema, hyperkeratosis, skin desquamation, and ulceration with various degrees of severity in the facial area, ears (Fig. 1), chest, thigh and neck (Fig. 2), which were compatible with photosensitization. There was fibrous plant material, pod fragments, *E. cyclocarpum* seeds, abundant gas and whitish foam material as well as erosions and moderate mucosa congestion in fore-stomachs (Figs. 3 and 4). Extensive mucosa erosions and multifocal moderate congestion were prominent in the abomasum and duodenum of T2 animals (Fig. 5). All livers of *E. cyclocarpum* exposed animals showed rounded edges and moderate dilatation of gallbladder, which was prominent in T3 animals (Fig. 6).

All microscopical lesions described had a statistical association with *E. cyclocarpum* pods intake. No microscopic alterations were found on encephalon. Table 3 indicates severity of main lesions. The control group showed no lesions in fore-stomachs (Fig. 7), whereas in the T2, T3 and T4 groups, microcirculatory changes were found in the digestive tract, which ranged from moderate to severe, characterized by edema and congestion of the microvasculature; likewise, inflammatory changes were found such as the presence of an inflammatory infiltrate with a predominance of macrophages and neutrophils with a generalized distribution in the mucosal tunica of the abomasum, duodenum and jejunum; additionally, foci of ulceration of the rumen mucosa with moderate inflammatory infiltrate with characteristics similar to those previously described were found (Fig. 8). The inflammatory infiltrate and ulcers were associated with increased doses of *E. cyclocarpum* pods in T3 and T4 groups.

The control group showed no hepatic lesions (Fig. 9), whereas heifers in T3 and T4 groups exposed to *E. cyclocarpum* pods showed severe panlobular cytoplasmic vacuolization in hepatocytes (Fig. 10), besides animals from T3 presented foci of portal fibrosis in liver associated to treatment (Fig. 11) hepatocytes individualization and necrosis involving clusters of hepatocytes (Fig. 12). Extensive foci of necrosis of hepatocytes were associated with increased doses of *E. cyclocarpum* fruits in T3 and T4 animals.

Animals exposed to *E. cyclocarpum* pods continually (T3 and T4) showed moderate to severe tegumentary lesions, skin desquamation, ulceration (Fig. 13), congestion and hemorrhages.

Kidney lesions such protein casts, tubular epithelium vacuolization and necrosis (Fig. 14) besides generalized congestion, were evident in animals fed with *E. cyclocarpum* with higher severity in T3 group.

Table 3
Severity of main microscopical lesions in Brahman heifers exposed to *E. cyclocarpum* fruits.

Lesions	Control (T1)	10 g/kg single dose (T2)	10 g/kg repeated dose (T3)	20 g/kg repeated dose (T4)
Gastrointestinal tract				
Mucosal mixed inflammatory infiltrate	0	1	3	2[a]
Atrofy and loss of villi	0	2[a]	1	1
Mucosal microvasculature changes (edema and congestion)	0	2[a]	1	1
Ruminal ulceration	0	1	3[a]	2[a]
Liver				
Portal fibrosis	0	1	2[a]	0
Intracytoplasmatic vacuolization of hepatocytes	0	1	3[a]	3[a]
Hepatocyte necrosis	0	0	2[a]	3[a]
Kidney				
Tubular epithelium vacuolization	0	1[a]	2[a]	1[a]
Protein in Bowman space and tubules	0	1[a]	2[a]	0
Tegumentary				
Congestion in superficial dermis	0	0	2[a]	2[a]
Hemorrhages	0	0	3[a]	2[a]
Ulceration	0	0	3[a]	2[a]

0: No lesion; 1: Mild; 2: Moderate; 3: Severe. Each number indicate average among 3 animals. Decimals were approximated to whole number.

[a] Indicate association between the treatments and the response variable ($p < 0.05$).

4. Discussion

This study for the first time shows that Brahman (*Bos indicus*) heifers exposed to different doses of *E. cyclocarpum* fruits in the feed developed digestive, tegumentary, and renal alterations with various degrees of severity. Other signs like prostration, muscle tremors and opisthotonos were associated to secondary alterations to main lesions. Experimental exposure of three cattle to *E. cortortisiliquum* (single dose of 5, 9 and 12 g/kg) reported similar lesions in the digestive tract (Mendonca et al., 2009), however, our study showed a broader spectrum of clinical signs such as foamy tympanism and those related to photosensitization, some not described in intoxication with *E. cyclocarpum* or with other plant species of the same genus.

Heifers exposed to a single dose of 10 g/kg of *E. cyclocarpum* pods developed mild and transient signs (tympanism, apathy, depression and hypophagia), however fifteen days after exposure animals still had paraclinical alterations (hyperfibrinogenemia, and urea elevation) and there were persistent lesions on rumen, liver and kidney. These finding suggest severity of lesions induced by *E. cyclocarpum* pods as animals could not return to basal state after two weeks. These bovines developed tympanism, whereas repeated doses of 10 and 20 g/kg caused severe tympanism, as well as photosensitization. Tympanism was observed in only one bovine exposed to a single dose (10 g/kg) of *E. contortisiliquum*, a plant that appear to cause a slightly different pattern of lesions in bovines where repeated doses ranging from 1.25 to 20 g/kg caused mainly diarrhea, liquid feces or death (Tokarnia et al., 1999).

Tympanism is related to several factors such as the high protein content and high palatability of *E. cyclocarpum* pods and the high content of saponins favors the production of foams (Wang et al., 2012). The fruit of *E. cyclocarpum* contain high concentrations of protein, carbohydrates and saponins that in the ruminal environment favor fermentation and gas accumulation (Wang et al., 2012). In addition, saponins bind minerals and electrolytes such as Na+, K+ and Ca++ that may form complexes that favor the retention of liquid and the death of entonidiomorphic protozoa that usually degrade chloroplasts, which accumulate and facilitate the formation of foams (Espinasse et al., 1995; Hess et al., 2003; Serratos et al., 2008). Atony related to tympanism yield to a ruminal dysbiosis that favored acid lactic producer bacteria (Kaneko et al., 2008) decreasing pH and supporting the ruminitis. As consequence an abomasum dysfunction with pyloric stenosis appears which would block electrolytic interchange (Cl^-/HCO_3^-) in duodenum and contributes to metabolic alkalosis (Kaneko et al., 2008). These electrolytic and acid base variations worse clinical signs, for example, those related to nervous system. In this study no macroscopic or microscopic lesions were found on encephalon that explain neurological signs (depression, opisthotonos or muscle tremors), so we assume that these alterations were caused by loss on acid base equilibrium or electrolytic and metabolic disorders; unfortunately, we did not measure these parameters. Besides, although animals exposed to *E. cyclocarpum* fruits developed liver injury and secondary photosensitization, neurological signs were not associated to hepatic encephalopathy as pathological lesions were not found on encephalon.

Skin lesions in animals exposed to repeated doses of *E. cyclocarpum* constitute a photosensitization of hepatic origin, as cited for hepatogenic photosensitization (Constable et al., 2016). Negrón et al. (1993)

reported photosensitization in cattle after the inclusion of 75% of *E. cyclocarpum* fruit in the diet. In addition, saponins from different *Enterolobium* species could induce hepatic injury (Francis et al., 2002). Besides photosensitization, some authors had reported that cattle exposed to *E. contortisiliquum* also developed severe vasculature changes, hepatic degeneration and jaundice (Mendonca et al., 2009 and Olinda et al., 2015), alterations that were found herein in heifers from T3 and T4 groups.

In this study, the *E. cyclocarpum* exposed animals showed a significant increase in GGT and AST enzymes activities associated with liver alteration. GGT is a cholestasis marker that points out canaliculi damage (Kaneko et al., 2008), which is supported by portal fibrosis found particularly on T2 group. AST is known to be indicative of hepatocyte injury (Villa et al., 1999). Although this enzyme is a muscle lesion marker too, and animals in the study presented tremors, is highly probable that AST elevation is merely associated with hepatic lesions according to clinical and pathological findings; CK activity measure would have clarify muscle lesion contribution to increased AST; however, under enzyme liberation from muscle, AST elevates in a proportional way to muscle body mass index (Kaneko et al., 2008) and its values would be greater that herein registered.

Cattle exposed to increased levels and frequency of *E. cyclocarpum* fruits (T3 and T4) showed polycythemia, a hematological change probably due to the dehydration generated by depression, no water intake or the inflammatory events occurring in various organs (Terra and Reynolds, 2021). In addition, in animals from T3 and T4 group urea and CREA increased from slight to moderate, this alteration in conjunction with hypovolemia and hence glomerular filtration rate reduction, trigger a prerenal azotemia which turn into renal azotemia because hypoxia or by direct action of substances present in *E. cyclocarpum*. Microscopic findings in kidney suggest that the consumption of *E. cyclocarpum* pods causes renal lesions, which have also been documented by others (Sowemimo et al., 2015).

The marked increase in serum fibrinogen may suggest increased synthesis during the initial phase of intoxication and it is consistent with a role of this protein as predictor of acute phase inflammation in cattle (Gruys et al., 2005). Besides, relative and absolute neutropenia observed on T4 would demonstrate neutrophil sequestration on different tissues, an additional support for acute inflammation in conjunction with histopathological findings on digestive system and skin.

Cattle exposed to higher doses (T3 and T4) of *E. cyclocarpum* fruit showed severe lesions in the liver, fore-stomachs, abomasum and intestine. It is known that saponins have an irritative effect on the mucosa of the gastrointestinal tract, a phenomenon that favors the synthesis of proinflammatory mediators from the arachidonic acid and chemotaxis of leukocytes (Pizzani et al., 2006). To our knowledge, microscopic findings of the digestive tract such as erosions in rumen, edema, congestion, haemorrhages, and mixed inflammatory infiltrate have not been described in toxic processes caused by *E. cyclocarpum* pods in cattle, and they may be associated with the irritant effect of saponins or ruminal acidosis similar to the ruminal lactoaciadosis in sheep caused by species of *E. contortisiliquum* (Pupin et al., 2017). Saponin crystals from *Brachiaria* sp., could be found in the lumen of the bile canaliculi in the liver (Faccin et al., 2014), however, they were no found in animals treated with *E. cyclocarpum* fuits. The ability of saponins to interact with the cell membrane could explain the wide range of lesions, like those reported in the cytotoxicity study carried out by Sowemimo et al. (2015), who demonstrated cell death in cell cultures 48 h after exposition to *E. cyclocarpum* extracts. Cell death was characterized by alterations in cell membrane integrity, DNA fragmentation, cell cycle arrest and induction of apoptosis by activation of caspase 3 (Sowemimo et al., 2015).

5. Conclusions

Brahman (*Bos indicus*) heifers exposed to *E. cyclocarpum* fruits under the agro-ecological conditions of the Eastern Plains of Colombia induced two clinical and pathological forms of forage poisoning: 1) A severe intoxication that affected the digestive system (bloat and diarrhea), skin (photosensitization), and kidney when repeated high (20 g/kg/d) or low (10 g/kg/d) doses were used, and 2) a mild to moderate form characterized by transient and different levels of tissue damage in the same organs/systems, when a single low dose (10 g/kg/d) was used; impairments that persisted two weeks after exposure. The high sensitivity of Brahman heifers to this poisonous plant suggests the need to restrict they access to *E. cyclocarpum* fruits or the improvement of extensive livestock production in this region of Colombia.

Ethical statement

Authors declare that all procedures were approved by the Bioethics Committee of the Faculty of Veterinary Medicine and Zootechnics of the National University of Colombia.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors want to thank to MINCIENCIAS (Ministry of Science, Technology, and Innovation) that funded this research through grant No. 1101-452-21116, to Héctor Julio Rincón, MVZ, Camilo Oicatá MVZ and Carlos Sánchez MVZ for technical support and to Gilberto Córdoba (Histotechnologist) and Leonardo Bernal (Laboratory Assistant).

References

Constable, P.D., Hinchcliff, K.W., Done, S.H., Gruenberg, W., 2016. Diseases of the skin, conjunctiva and external ear. In: Constable, P.D., Hinchcliff, K.W., Done, S.H., Gruenberg, W., Radostits, O.M. (Eds.), Veterinary Medicine - A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats, eleventh ed. Elsevier, St Louis Missouri, pp. 1540–1660.

Costa, R.L.D., Marini, A., Tanaka, D., 2009. Um caso de de intoxicação de bovinos por *Enterolobium contortisiliquum* (timboril) no brasil. Arch. Zootec. 58, 313–316.

Espinasse, J., Kuiper, R., Schelcher, F., 1995. Physiopathologie du complexe gastrique. In: Jarrige, R., Ruckebusch, Y., Demarquilly, C., Farce, M.H., Journet (Eds.), Nutrition des ruminants domestiques — ingestion et digestion. INRA, Paris, pp. 805–852.

Faccin, T., Riet-Correa, F., Rodriguez, F.S., Santos, A., Melo, G.K.A., Silva, J., Ferreira, R., Itavo, C., Lemos, R.A., 2014. Poisoning by *Brachiaria brizantha* in flocks of naïve and experienced sheep. Toxicon 82, 1–8. https://doi.org/10.1016/j.toxicon.2014.02.008.

Francis, G., Kerem, Z., Makkar, H.P., Becker, K., 2002. The biological action of saponins in animal systems: a review. Br. J. Nutr. 88, 587–605. https://doi.org/10.1079/BJN2002725.

Grecco, F.B., Dantas, A.F., Riet-Correa, F., Leite, C.G., Raposo, J.B., 2002. Cattle intoxication from *Enterolobium contortisiliquum* pods. Vet. Hum. Toxicol. 44, 160–162.

Gruys, E., Toussaint, M.J.M., Niewold, T., Koopmans, S.J., 2005. Acute phase reaction and acute phase proteins. J. Zhejiang Univ. - Sci. B. 6, 1045–1056. https://doi.org/10.1631/jzus.2005.B1045.

Hess, H.D., Kreuzer, M., Díaz, T.E., Lascano, C.E., Carulla, J.E., Soliva, C.R., Machmüller, A., 2003. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. Anim. Feed Sci. Technol. 109, 79–94. https://doi.org/10.1016/S0377-8401(03)00212-8.

ICA Censo Pecuario Nacional, 2021. Censo bovino en Colombia. https://www.ica.gov.co/areas/pecuaria/servicios/epidemiologia-veterinaria/censos-2016/censo-2018.

Kaneko, J., Harvey, W., Bruss, M., 2008. Clinical Biochemistry of Domestic Animals, sixth ed. Elsevier, USA. Burlington.

Lozano, M.C., Doncel, B., Moreno, C.A., 2011. Manual de plantas tóxicas para bovinos. Región Llanos Orientales de Colombia: Meta y Casanare. Universidad Nacional de Colombia, Bogotá, Colombia.

Luna, L., 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Company, New York, USA.

Mello, G.W.S., Oliveira, D.M., Carvalho, C.J.S., Pires, L.V., Costa, F.A.L., Riet-Correa, F., Silva, S.M.M., 2010. Plantas tóxicas para ruminantes e eqüídeos no Norte Piauiense. Pesqui. Vet. Bras. 30, 1–9. https://doi.org/10.1590/S0100-736X2010000100001.

Mendonca, F.S., Evencio-Neto, J., Baratella-Evencio, B., Doria, R.G., 2009. Natural and experimental poisoning of cattle by *Enterolobium contortisiliquum* pods (Fabaceae

mimosoideae) in Central-Western Brazil. Acta Vet. 78, 621–625. https://doi.org/10.2754/avb200978040621.
Mingatto, F.E., Santos, A.B., Lupatini, G.C., 2008. Intoxicação por *Enterolobium contortisiliquum* em bovinos no município de Pacaembu. SP. Pubvet. 2, 18.
Negrón, G., Parra, O., Avila, N., Hoet, A., 1993. Efecto experimental del fruto del *Enterolobium cyclocarpum* (Kara-Kara) en el ganado bovino. Rev. Cient. FCV-LUZ. 3, 1–6.
Olfert, E., Cross, B., McWilliam, A., 1993. Guide to the Care and Use of Experimental Animals, 2th ed., vol. 1. Canadian Council of Animal Care, Ottawa, Canada.
Olinda, R.G., Medeiros, R.M.T., Dantas, A.F.M., Lemos, R.A.A., Riet-Correa, F., 2015. Intoxicação por *Enterolobium contortisiliquum* em bovinos na região Nordeste do Brasil. Pesqui. Vet. Bras. 35, 44–48. https://doi.org/10.1590/S0100-736X2015000100010.
Pizzani, P., Matute, I., De Martino, G., Arias, A., Godoy, S., Pereira, L., Palma, J., Rengifo, M., 2006. Composición Fitoquímica y Nutricional de Algunos Frutos de Árboles de Interés Forrajero de Los Llanos Centrales de Venezuela. Rev. Fac. Cienc. Vet. 47, 105–113.
Pupin, R.C., Leal, P.V., Lima, S.C., Melo, G.K.A., Pott, A., Araújo, A., Barros, C.S.L., Lemos, R.A.A., 2017. *Enterolobium contortisiliquum* is a cause of acute ruminal acidosis in sheep. Toxicon 126, 90–95. https://doi.org/10.1016/j.toxicon.2016.12.015.
R core team, 2017. R: a Language and Environment for Statistical Computing. R Foundation for Statistical computing, Viena. www.R-project.org/.
Riet-Correa, F., Medeiros, R.M.T., 2001. Intoxicações por plantas em ruminantes no Brasil e no Uruguai: importância econômica, controle e riscos para a saúde pública. Pesqui. Vet. Bras. 21, 38–42. https://doi.org/10.1590/S0100-736X2001000100008.
Serratos, J.C., Carreón, J., Castañeda, H., Garzón, P., García, J., 2008. Composición químico-nutricional y de factores antinutricionales en semillas de parota *(Enterolobium cyclocarpum)*. Interciencia 33, 850–854.
Shaw, F.D., Reilly, J.S., 2001. Cattle. In: Reily, J.S. (Ed.), Euthanasia of Animals Used for Scientific Purposes. ANZCCART, Adelaide, pp. 49–51. http://www.adelaide.edu.au/ANZCCART/docspublications/eEuthanasia.pdf.
Sowemimo, A., Venables, L., Odedeji, M., Koekemoer, T., van de Venter, M., Hongbing, L., 2015. Antiproliferative mechanism of the methanolic extract *Enterolobium cyclocarpum* (Jacq.) Griseb. (Fabaceae). J. Ethnopharmacol. 159, 257–261. https://doi.org/10.1016/j.jep.2014.11.023.
Terra, R.L., Reynolds, J.P., 2021. Ruminant history, physical examination, welfare assessment and records. In: Smith, B.P., Van Metre, C., Pusterla, N. (Eds.), Large Animal Internal Medicine, sixth ed. Elsevier, St. Louis, Missouri, pp. 2–13.
Tokarnia, C.H., Döbereiner, J., Dutra, I.S., Brito, I.S., Chagas, B.R., Franca, T.N., Brust, L. A.G., 1999. Experimentos em bovinos com as favas de *Enterolobium contortisiliquum* e *E. timbouva* para verificar propiedades fotossensibilizantes e/ou abortivas. Pesqui. Vet. Bras. 19, 39–45. https://doi.org/10.1590/S0100-736X1999000100006.
Tokarnia, C.H., Dobereiner, J., Vargas, P., 2002. Poisonous plants affecting livestock in Brazil. Toxicon 40, 1635–1660. https://doi.org/10.1016/S0041-0101(02)00239-8.
Torres, J.E., Echeverry, R., Mejía, B., 1983. Cromatosis de los bovinos o “vaca morada” en Colombia. Carta Ganad. 20, 25–30.
Villa, N.A., Ceballos, A., Ceron, D., Serna, C.A., 1999. Valores bioquímicos sanguíneos en hembras Brahman bajo condiciones de pastoreo. Pesqui. Agropecu. Bras. 34, 2339–2343. https://doi.org/10.1590/S0100-204X1999001200021.
Wang, Y., Majak, W., McAllister, T.A., 2012. Frothy bloat in ruminants: cause, occurrence, and mitigation strategies. Anim. Feed Sci. Technol. 172, 103–114. https://doi.org/10.1016/j.anifeedsci.2011.12.012.