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Experimental poisoning by *Brachiaria decumbens* in rabbits[1]

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ABSTRACT.- Utiumi K.U., Albuquerque A.S., Burque A.S., Souza F.R., Sonne L., Varaschin M.S., Raymundo D.L. & Peconick A.P. 2018. **Experimental poisoning by *Brachiaria decumbens* in rabbits.** *Pesquisa Veterinária Brasileira 38(10):1885-1889*. Setor de Medicina Veterinária Preventiva, Departamento de Medicina Veterinária, Universidade Federal de Lavras, Cx. Postal 3037, Lavras, MG 37200-000, Brazil. E-mail: ki_uemura@yahoo.com.br

Brachiaria spp. are important sources of forage for ruminants in Brazil, due to the easy cultivation, good resistance to drought, good adaptation to different soils and low maintenance cost. However, the ingestion of this grass has been related to photosensitization outbreaks in cattle and sheep with significant economic losses. The hepatotoxic effects related to the ingestion of grass are the formation of crystals and foamy macrophages due to the accumulation of toxic metabolites. The use of cattle and sheep in experiments involving the plant presents several obstacles in the ethical, economic and animal management. The objective of this study was to evaluate the sensitivity of rabbits as an experimental model for *B. decumbens* poisoning. Two experiments were carried out. In Experiment 1 four rabbits received the fresh plant in daily doses of 10, 20, 40 and 80g/kg body weight for 120 days. In Experiment 2 three rabbits received the fresh plant in amounts of 500g daily with duration of 210 days. The animals of Experiment 1 showed no clinical signs and no macroscopic and microscopic changes characteristic of *B. decumbens* poisoning. In Experiment 2 the animals also showed no clinical signs or significant macroscopic alterations. Histological analysis showed isolated foamy macrophages or present in random groups of cells in the liver and mesenteric lymph nodes. Samples of liver and mesenteric lymph nodes of the rabbits of Experiment 2 were submitted to the lectin-histochemistry technique. The WGA, sWGA and RCA lectins showed reactivity in foamy macrophages in both organs. This is the first study of our knowledge that demonstrates histopathological lesions caused expetimentally by *Brachiaria* spp. in rabbits, demonstrating its potential as an animal model.

INDEX TERMS: Experimental model, hepatogenic photosensitization, lectin histochemistry, foamy macrophages, toxicoses.

RESUMO.- [**Intoxicação experimental por *Brachiaria decumbens* em coelhos.**] *Brachiaria* ssp. são importantes fontes de forragem para ruminantes no Brasil, devido ao fácil cultivo, boa resistência a seca, boa adaptação a diferentes solos e baixo custo de manutenção. Entretanto, a ingestão desta gramínea está relacionada a surtos de fotossensibilização, em bovinos e ovinos, principalmente, ocasionando prejuízos econômicos significativos. Os efeitos hepatotóxicos relacionados à ingestão da gramínea são a formação de cristais e macrófagos espumosos causados pelo acúmulo de metabólitos tóxicos. A utilização de bovinos e ovinos em experimentos envolvendo a planta apresenta vários empecilhos, tanto no âmbito ético, econômico e no manejo dos animais. O objetivo do presente trabalho foi avaliar a sensibilidade de coelhos como modelo experimental para intoxicação por *B. decumbens*. No presente estudo foram realizados dois experimentos. O Experimento

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1 utilizou quatro coelhos que receberam a planta fresca em doses diárias de 10, 20, 40 e 80 g/Kg de peso vivo durante 120 dias. O Experimento 2 utilizou três coelhos recebendo a planta fresca em quantidades de 500g diárias por animal com duração de 210 dias. No Experimento 1, os animais não apresentaram sinais clínicos e nem alterações macroscópicas e microscópicas características de intoxicação por *B. decumbens*. No Experimento 2 os animais também não apresentaram sinais clínicos e alterações macroscópicas significativas. Na análise histológica observou-se presença de macrófagos espumosos isolados ou em grupos aleatórios de células no fígado e nos linfonodos mesentéricos. Amostras de fígado e linfonodos mesentéricos dos animais do Experimento 2 foram submetidos à técnica de lectino-histoquímica. As lectinas WGA, sWGA e RCA apresentaram reatividade em macrófagos espumosos nos dois órgãos. Este é o primeiro trabalho de nosso conhecimento que demonstra lesões histopatológicas por *Brachiaria spp* conduzido de forma experimental em coelhos, demonstrando seu potencial como modelo animal nesse campo de estudo.

TERMOS DE INDEXAÇÃO: Modelo experimental, fotossensibilização hepatógena, lectino-histoquímica, macrófago espumoso, toxicoses.

INTRODUCTION

Most of the pasture lands in Brazil correspond to *Brachiaria* spp. populations, mainly *B. decumbens* and *B. brizantha*, considered important forage sources for ruminants (Assis et al. 2003). However, annual outbreaks of photosensitization caused by the ingestion of these grasses are reported in several species of animals, especially cattle, sheep (Brum et al. 2007, Boabaid et al. 2011), goats and buffaloes, and eventually in equines (Barbosa et al. 2006, Riet-Correa et al. 2011, Miranda et al. 2016). Consequently, they cause significant economic losses, as well as a decrease in the productivity of Brazilian herds (Tokarnia et al. 2012).

The hepatotoxicity of *Brachiaria* is caused by protodioscin, a lithogenic steroidal saponin present in the plants (Brum et al. 2007, Riet-Correa et al. 2011, Miranda et al. 2016). Protodioscin also causes obstruction of bile ducts and the accumulation of phylloerythrin and consequent photosensitization with skin lesions (Lemos et al. 1997, Driemeier et al. 1999, Brum et al. 2007, Santos Junior 2008, Mustafa et al. 2012, Tokarnia et al. 2012). However, the exact amount of *Brachiaria* spp. that has to be ingested to cause photosensitization is still unknown (Mendonça et al. 2008).

Lectins are proteins or glycoproteins with one or more subunit binding sites that can reversibly bind to specific carbohydrate residues, agglutinate cells and/or precipitate polysaccharides or glycoproteins (Costa et al. 2013). Lectin-histochemistry can be used for the diagnosis of the hepatotoxicity of *Brachiaria*. The technique is efficient to detect carbohydrate residues that accumulate in foamy macrophages in bovine tissues that consume *Brachiaria* spp. (Costa et al. 2013).

Some animal species are used in experimental poisoning. Among them, sheep (Driemeier et al. 2002) present some advantages over cattle, including lower maintenance costs and easier management (Thompson et al. 1983). The use of other experimental models, such as rabbits, brings even more advantages because they are easy to manage and maintain, besides having a lower cost and faster reproductive cycle (Thompson et al. 1983). The few experiments on *Brachiaria* spp. poisoning in rabbits conducted so far have demonstrated that the plant is palatable to the animals, but the studies did not successfully reproduced lesions and clinical signs (Souza et al. 2012, Facin et al. 2016).

The objective of this study was to create an experimental model using rabbits for experimental poisoning by *B. decumbens* resulting in typical hepatic lesions as seen in cattle and sheep.

MATERIALS AND METHODS

The experiment was approved by the Ethics Committee on Animal Use (ECAU) of UFLA under protocol number 078/2013. Sixty-day old male rabbits of the New Zealand breed were used in the study. The rabbits were housed in individual cages with water and commercial feed *ad libitum*. The plant access of *Brachiaria decumbens* Stapf cv Common was collected in the Department of Veterinary Medicine of the Federal University of Lavras (21°13'52"S and 44°58'32"W) in morning hours. An exsiccate of the species was deposited in the University Herbarium for consultation.

Fresh plants were collected daily in the same field and offered to the animals; the non-consumed surplus was weighed and then discounted from the total value received. Two different experiments were carried out: in Experiment 1, four rabbits were kept in individual cages and given the amounts of 10g/kg for Rabbit 1; 20g/kg for Rabbit 2; 40g/kg for Rabbit 3; and 80g/kg for Rabbit 4. All rabbits were fed with fresh *B. decumbens*. Rabbits were fed and monitored daily during 120 days; after that, they were euthanized and necropsied.

In Experiment 2, we used three rabbits. Each animal received 500g of *B. decumbens* daily, the non-consumed surplus was weighed and discounted from the 500g of food provided daily. This experiment lasted 210 days; after this time, the rabbits were euthanized and necropsied.

In both experiments, fragments of various organs were collected and fixed in 10% buffered formalin solution, routinely processed for histopathology with sections stained with hematoxylin and eosin (HE). The liver and lymph node fragments of animals from Experiment 2 were submitted to the lectin-histochemistry technique. The lectins used were *Canavalia ensiformis* agglutinin (Con-A), *Dolichos biflorus* agglutinin (DBA), *Glicine max* agglutinin (SBA), *Arachis hypogaea* agglutinin (PNA), *Riccinus communis* agglutinin-I (RCA-I), *Ulex europaeus* agglutinin-I (UEA-I), *Triticum vulgaris* agglutinin (WGA), Succinyl-WGA (sWGA), *Griffonia* (Bandeiraea) *simplicifolia (GSL), Sophora japônica* (SJA), *Pisum sativum* (PSA), *Phaseolus vulgaris* (PHA-L and PHA-E) and *Lens culinaris* or *Lens esculenta* (LCA). The histochemical lectin technique used was the same as that one reported by Boabaid et al. (2011).

RESULTS

The plant was identified as *Brachiaria decumbens* and an exsiccate was deposited for botanical confirmation and reproducibility of the present work.

In Experiment 1, the animals did not present clinical signs such as weight reduction, photosensitization or behavior change, and no macroscopic or microscopic alterations typical of *B. decumbens* poisoning were found. The daily toxic dose spontaneously ingested by each rabbit was 52g/kg in Rabbit 1; 78g/kg in Rabbit 2; 61g/kg in Rabbit 3; and 133g/kg in Rabbit 4.

In Experiment 2, the animals showed no clinical signs such as weight reduction, photosensitization or behavior change

and significant macroscopic alterations. In the histological analysis, we observed foamy macrophages isolated or in random groups of cells in the middle of the hepatic cords (Fig.1 and 2); hepatocyte swelling and vacuolization; individual hepatocyte necrosis; mild proliferation of bile ducts and proliferation of periportal connective tissue associated with periportal mononuclear infiltrates. In isolated mesenteric lymph nodes, isolated macrophages were seen in lymphatic sinuses (Fig.3) and follicular centers. The daily amount ingested by each rabbit in Experiment 2 was 203g/kg in Rabbit 5, 191g/kg in Rabbit 6, and 192g/kg in Rabbit 7.

Liver and mesenteric lymph node samples of the animals of Experiment 2 subjected to lectin-histochemistry are summarized in Table 1. The lectins sWGA (Fig.4), RCA-I, WGA, and GSL showed high reactivity and specificity for foam macrophages present in the liver of rabbits poisoned by *Brachiaria decumbens.* The lectins Con-A, PNA and PHA-L showed moderate reactivity and specificity in liver foam macrophages. The foamy macrophages observed in mesenteric lymph nodes presented slight reactivity and specificity for sWGA (Fig.5), RCA-I, WGA, Con-A, PNA, PHA-L and PHA-E.

DISCUSSION

After the two experiments were carried out, it was possible to reproduce the hepatic and lymph node lesions in rabbits in Experiment 2. These lesions are similar to those described in cases of *Brachiaria decumbens* poisoning in sheep (Lemos et al. 1996) and cattle (Driemeier et al. 1998). The most characteristic injury caused by *Brachiaria* spp. poisoning is the presence of birefringent crystals and macrophages in bile ducts (Riet Correa et al. 2011), as wellas foamy

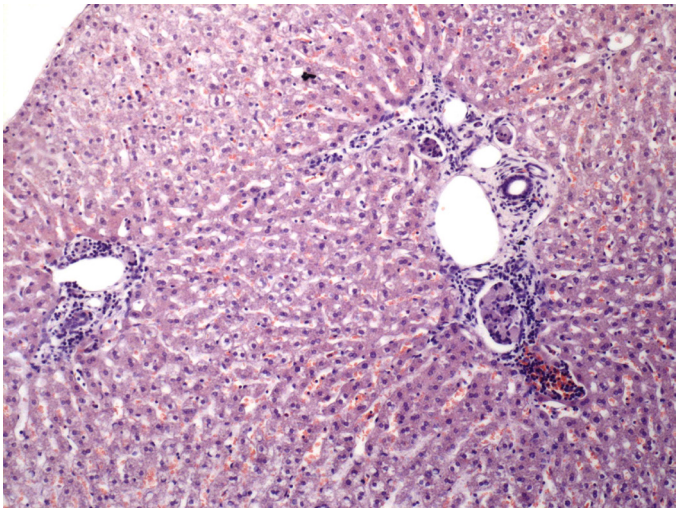

Fig.1. Rabbit liver experimentally poisoned by *Brachiaria decumbens*. Numerous isolated or clustered macrophages are observed. HE, obj.10x.

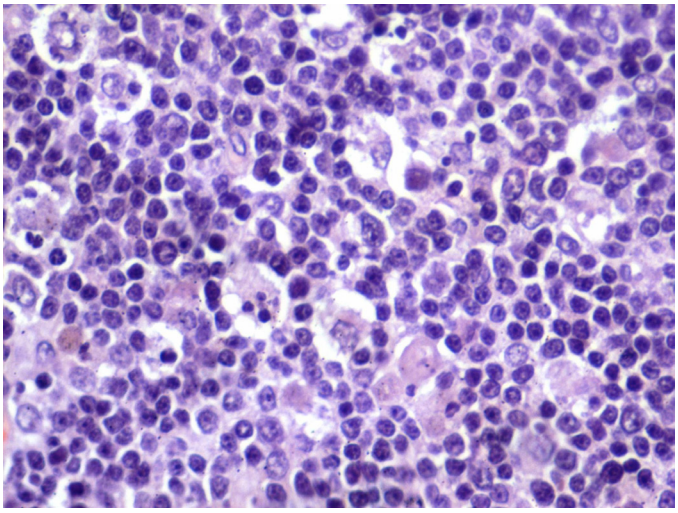

Fig.3. Rabbit mesenteric lymph node experimentally poisoned by *Brachiaria decumbens*. Isolated foamy macrophages are seen in lymphatic sinuses. HE, obj.40x.

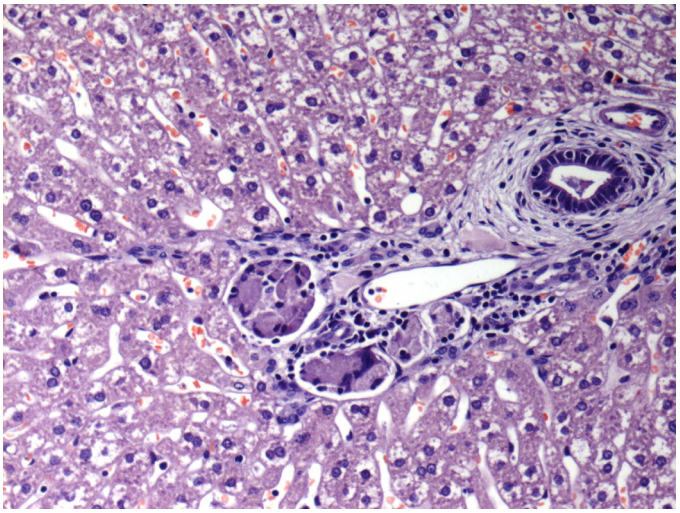

Fig.2. Rabbit liver experimentally poisoned by *Brachiaria decumbens.* Numerous isolated or clustered macrophages are observed. HE, obj.20x.

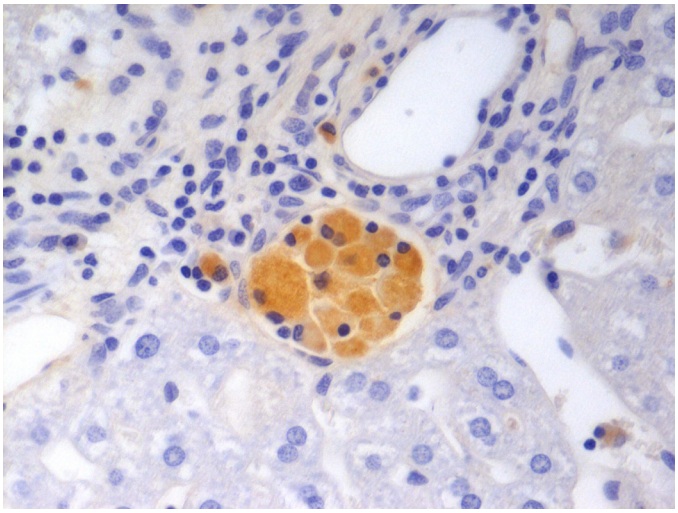

Fig.4. Rabbit liver experimentally poisoned by *Brachiaria decumbens*. Marked labeling for Succinyl-WGA (sWGA) in groups of foamy macrophages. Lectin-histochemistry, obj.40x.

Table 1. Lectin-histochemical evaluation of the liver and mesenteric lymph node of rabbits experimentally poisoned with *Brachiaria decumbens*

	Macrophages	Hepatocytes	Bile duct	Bile duct contents	Bile duct surface	Intimate vase	Connective tissue	Lymph node
Con-A	++*	++	++	-	-	++	-	Macrophage +
DBA	-	-	+	-	-	-	-	-
SBA	+	+	-	-	-	-	-	-
PNA	++	-	+	+	-	-	-	Macrophage +
RCA-I	++/+++	+	++	+++	+	+++	-	Macrophage +
UEA-I	-	-	++	++	-	-	-	-
WGA	++/+++	-	-	+	-	+	++	Macrophage +
sWGA	+++	-	-	-	-	-	-	Macrophage +
GSL	++/+++	-	+	-	-	-	-	-
SJA	-	-	-	-	-	-	-	-
PSA	-	-	-	-	-	+	+	-
PHA-L	+/++	-	+	-	-	-	-	Macrophage +
PHA-E	+	-	-	-	-	-	-	Macrophage +
LCA	-	-	-	-	-	-	-	-

* +++ Acute injury, ++ moderate injury, + mild injury, - no injury.

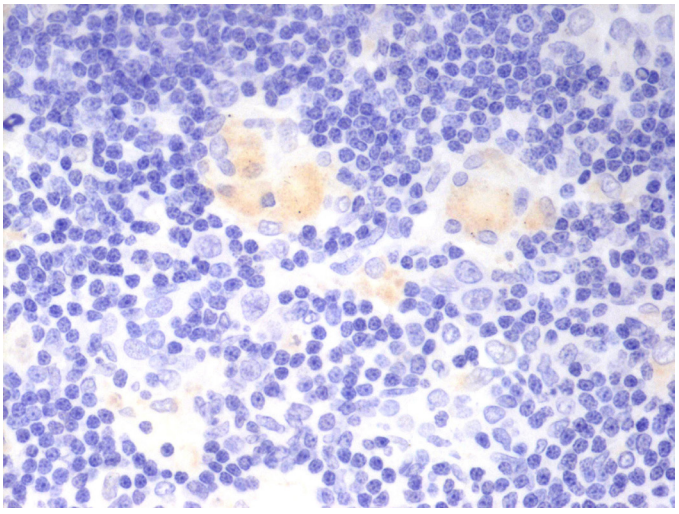

Fig.5. Rabbit mesenteric lymph node experimentally poisoned by *Brachiaria decumbens*. Light labeling for Succinyl-WGA (sWGA) in foamy macrophages isolated on lymphoid follicles. Lectin-histochemistry, obj.40x.

macrophages alone or in randomized groups in the liver (Lemos et al. 1996, Driemeier et al. 1998, Riet-Correa et al. 2010). The presence of foamy macrophages in the liver, either alone or in random groups, was the most consistent finding of *B. decumbens* poisoning in rabbits of Experiment 2; however, in no animal birefringent crystals in bile ducts and macrophages were present, which is also described in other cases (Riet-Correa et al. 2010, Miranda et al. 2016). The presence of foamy macrophages in mesenteric lymph nodes is described in cases of poisoning in different ruminant species, both in lymphatic sinuses (Driemeier et al. 1998, Riet-Correa et al. 2010) and follicular centers (Boabaid et al. 2011, Miranda et al. 2016).

Experiment 1 demonstrated that daily toxic doses of up to 133g/kg of body weight for up to 120 days are not enough to cause hepatic lesions by *Brachiaria decumbens* poisoning. These findings are similar to those of another study using rabbits as an experimental model for disease which also demonstrated that lower doses and times did not cause clinical signs or lesions in rabbits (Facin et al. 2016). Experiment 2 demonstrated that daily toxic doses of 190g/kg during 210 days were enough to induce hepatic lesions by *B. decumbens* poisoning.

The lectin-histochemistry labeling presented by rabbits poisoned by *B. decumbens* showed a pattern similar to other species. The marked labeling of foamy macrophages in the liver was seen mainly in the lectins RCA-I, WGA, sWGA and GSL, which was also observed in sheep (Boabaid et al. 2011) and buffaloes (Miranda et al. 2016). Slight labeling in the lectins Con-A-1, PNA and PHA-L was observed in the present study, as well as reported in buffaloes (Miranda et al. 2016) and ovines with more intensity (Boabaid et al. 2011, Costa et al. 2013). In the case of foamy macrophages in lymph nodes, the lectins Con-A, PNA, WGA, sWGA, PHA-L and PHA-E were slightly labeled in rabbits; while in sheep and buffaloes they had a moderate to intense labeling (Boabaid et al. 2011, Miranda et al. 2016).

There is only one reference in the literature of hepatic lesions containing foamy macrophages, but this occurred in a natural outbreak of *B. decumbens* poisoning in rabbits kept on pasture (Souza et al. 2012). The present experimental study, as well as another similar study (Facin et al. 2016), showed that rabbits present a higher resistance to *B. decumbens* poisoning because doses up to 130g/kg during a period of ingestion of up to 120 days were not enough to induce lesions in the liver or lymph nodes of the animals. Although lesions were observed in animals that ate doses of 190g/kg over a period of 210 days, it was not possible to define the toxic dose and minimum period necessary to observe hepatic lesions of poisoning.

The observation of development of lesions in the liver and lymph nodes in rabbits brings advances, both ethically and

economically, due to the possibility to replace other species, such as cattle and sheep, used in the study of *B. decumbens* poisoning. Furthermore, it is possible to advance the knowledge on the pathogenesis of the disease, highlighting the main determinants involved in plant poisoning, and also the better understanding of the effects of poisoning in horses because there are anatomical similarities of the digestive tract between the two species.

CONCLUSION

The results demonstrate that it is possible to use rabbits as models for studies of experimental poisoning by *Brachiaria decumbens*; it was possible to reproduce liver lesions with the presence of foamy macrophages and also in lymph nodes, similar to lesions found in cattle, sheep and horses.

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