Pathological Effects of Short-Term *Crotalaria retusa* Ingestion by Guinea Fowl (*Numida meleagris*)

Windleyanne Gonçalves Amorim Bezerra, Milzete Alves de Souza, Ruben Horn Vasconcelos, Luis Augusto Vieira Cordeiro, Jael Soares Batista, and Benito Soto-Blanco

Department of Animal Sciences, Federal Rural University of Semi-Árido (UFERSA), Mossoró, RN, Brazil

Correspondending author: B. Soto-Blanco, soto-blanco@ufersa.edu.br

Abstract

Crotalaria retusa and its seeds contain toxic pyrrolizidine alkaloids that can contaminate feeds and food, thus poisoning livestock and humans. The objective of this work was to determine the toxic effects of the ingestion of *Crotalaria retusa* seeds by guinea fowl (*Numida meleagris*). Eighteen young guinea fowl were randomly assigned to 3 groups and treated with 0 (control), 1.0 mg/kg or 5.0 mg/kg of crushed *Crotalaria retusa* seeds mixed into the daily ration for 7 consecutive days. After 7 days, blood samples were collected to determine serum glucose, cholesterol, total proteins, urea and creatinine concentrations, and ALT and AST activities. After sampling, the guinea fowl were euthanized and necropsied, and samples of liver, kidneys, lungs, and heart were collected and prepared for histologic studies. At necropsy all animals treated with 5.0 mg/kg of *C. retusa* presented mild ascites (clear fluid), the liver were uniformly pale, icteric, and soft, and the gallbladders were distended and filled with bile, but the bile aspect and the gall bladder wall were normal. The histopathological examination of the livers from *C. retusa*-treated animals revealed centrilobular swollen and vacuolated hepatocytes, moderate in guinea fowl treated with 5.0 mg/kg and mild in those treated with 1.0 mg/kg. One animal treated with 5.0 mg/kg presented centrilobular necrosis and hepatomegalocytosis with prominent nucleolus. The histological examination of kidneys revealed vacuolated epithelial cells at distal convoluted tubules. These findings indicate that guinea fowl are very sensitive to poisoning by *Crotalaria retusa*.

Keywords: Poisonous plants, pyrrolizidine alkaloids, monocrotaline, avian

Introduction

Plant species from the genus *Crotalaria*, including *C. retusa* (wedge-leaf rattlebox), are used as soil builders and green manure (Williams and Molyneux 1987) and for management of plant-parasitic nematodes (Thoden and Boppré 2010). However, these plants contain pyrrolizidine alkaloids (PAs), mainly monocrotaline (Williams and Molyneux 1987). *C. retusa* poisoning has been described in several species, including horses (Nobre et al. 2004), sheep (Nobre et al. 2005), pigs (Hooper and Scanlan 1977), chickens (Hooper and Scanlan 1977, Alfonso et al. 1993, Hatayde et al. 2008), and geese (Alfonso et al. 1993). Poultry are frequent victims of poisoning by such contamination under field conditions (Williams and Molyneux 1987); however, other avian species may often be affected. In addition to intentional ingestion of the plant, avians are poisoned by *Crotalaria* seeds through contamination of corn, sorghum, and soybean during harvest (Nakage et al. 2000).

There is a great variation in susceptibility to PA toxicity between animals of different species. This variation is a result of many different factors including differing rates of metabolic activation, hydroxylation, N-oxidation, hydrolysis, and glucuronidation of PAs (Huan et al. 1998, He et al. 2010). This objective of this study was to determine the pathological changes produced by the ingestion of *Crotalaria retusa* seeds by guinea fowl (*Numida meleagris*) for 7 days.

Material and Methods

Seeds from *Crotalaria retusa* L. were collected at Mossoró city, RN, northeastern Brazil (5°11'15"S and 37°20'39"W), at an altitude of 16 m above sea level. The climate in this region is characterized as semi-arid. The mean annual temperature in this region is 27.4°C, and the mean annual rainfall and mean relative humidity are 674 mm and 68.9%, respectively. The botanical identification was performed by Prof. Odaci Fernandes de Oliveira, Mossoró, RN, Brazil. Only mature seeds without any sign of lesion were used, and they were dried at room temperature and then powdered before use.

Eighteen 3- to 5-day-old guinea fowl (*N. meleagris*) were housed one animal per cage with free access to food and water. The fowl were randomly divided into 3 groups of 6 and treated with 0 (control), 1.0 mg/kg BW, or 5.0 mg/kg BW crushed *C. retusa* seeds that were mixed into their daily ration for 7 consecutive days. After treatment, blood samples were collected from all the guinea fowl from the jugular vein and serum was collected for a complete biochemical panel. The serum concentrations of albumin, cholesterol, urea, and uric acid, and the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using commercially available kits (Katal, Belo Horizonte, MG, Brazil) and an automatic analyzer SBA-2000 (Celm, Barueri, SP, Brazil) using recommended reagents and following the manufacturer's directions. Immediately after blood collection, the guinea fowl were euthanized and necropsied. At necropsy all the gross lesions were noted and small fragments of liver, kidney, and heart were collected, fixed in buffered formalin, imbedded in paraffin, and processed for routine histologic studies.

The study used a completely randomized design with three treatments. Statistical analyses were performed by analysis of variance (ANOVA) plus a posthoc Dunnett's test using the software BioEstat 5.0. Statistical significance was set at $P < 0.05$. Results are presented as means with standard errors.

Results

During the experimental period mortality occurred in all groups, two in the control group and those treated with 1.0 mg/kg of *C. retusa* and three in the group treated with 5.0 mg/kg of *C. retusa*. Clinical signs were observed in guinea fowl treated with 5.0 mg/kg of *C. retusa*, and consisted of anorexia, emaciation, muscular weakness, trembling, and depression in the 8 hours before death. Fowls from other groups presented anorexia before death, and the necropsy revealed congestion of liver, lungs, and intestines. There were no significant differences between the various biochemical parameters (table 1).

At necropsy all animals treated with 5.0 mg/kg of *C. retusa* presented mild ascites (clear fluid); the livers were uniformly pale, icteric, and soft; and the gall bladders were distended and filled with bile but the bile aspect and the gall bladder wall were normal. The necropsy of the other animals (controls and those treated with 1.0 mg/kg) killed at the end of the experiment revealed no gross lesion.

Table 1. Serum biochemical panel from guinea fowl (*Numida meleagris*) treated with 0 (control), 1.0 (G1.0), or 5.0 (G5.0) mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days

Parameter	Control	G1.0	G5.0
ALT (U/l)	11.3±5.46	10.0±1.67	13.0±8.13
AST (U/l)	360.1±68.6	407.5±87.7	313.2±105.9
Urea (mg/dl)	4.65±1.47	0.80±1.60	6.13±5.38
Uric acid (mg/dl)	7.94±1.15	6.15±1.98	9.24±1.80
Cholesterol (mg/dl)	136.1±43.5	162.3±30.3	138.3±43.7
Albumin (g/dl)	0.93±0.20	1.12±0.10	0.94±0.20

Data are presented as mean±SEM.
ALT: alanine aminotransferase. AST: aspartate aminotransferase.

Table 2. Pathological findings in guinea fowl (*Numida meleagris*) treated with 0 (control), 1.0 (G1.0), or 5.0 (G5.0) mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days

Lesion	Control	G1.0	G5.0
Centrilobular swollen and vacuolated hepatocytes	-	+	++
Centrilobular necrosis	-	-	++ [1]
Hepatomegalocytosis	-	-	+ [1]
Vacuolated renal tubular epithelial cells	-	+	+ / ++

- No lesion + mild ++ moderate +++ severe
[1] present in just one animal.

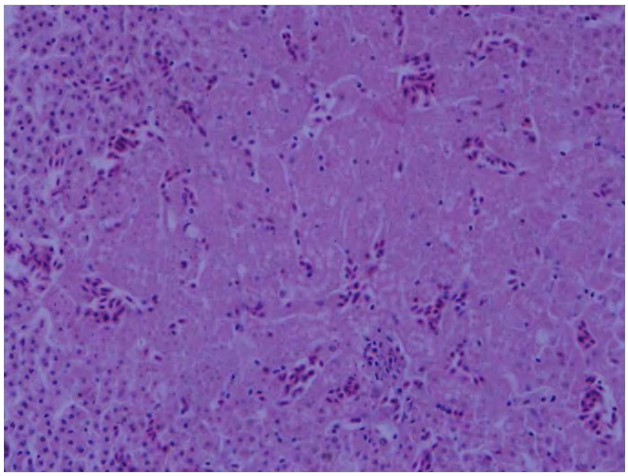

Figure 1. Liver of guinea fowl (*Numida meleagris*) treated with 5.0 mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days, showing vacuolated hepatocytes (HE, 40x).

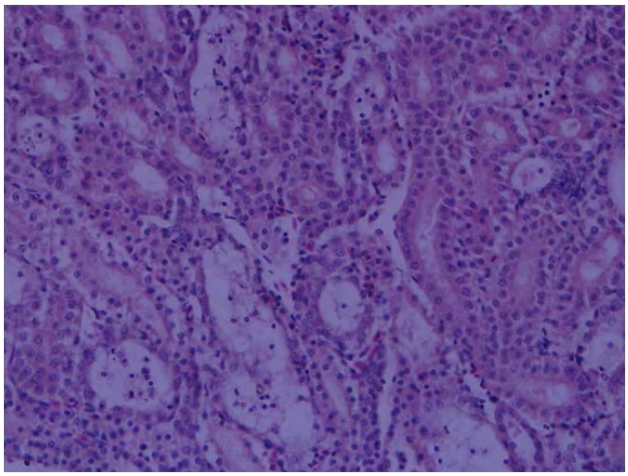

Figure 2. Liver of guinea fowl (*Numida meleagris*) treated with 5.0 mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days, showing centrilobular necrosis (HE, 40x).

The histopathological examination (table 2) of the livers from *C. retusa*-treated animals revealed centrilobular swollen and vacuolated hepatocytes (figure 1), moderate in guinea fowl treated with 5.0 mg/kg and mild in those treated with 1.0 mg/kg. One animal treated with 5.0 mg/kg BW presented centrilobular necrosis (figure 2) and hepatomegalocytosis with prominent nucleolus. The histological examination of kidneys revealed vacuolated epithelial cells at distal convoluted tubules (figure 3).

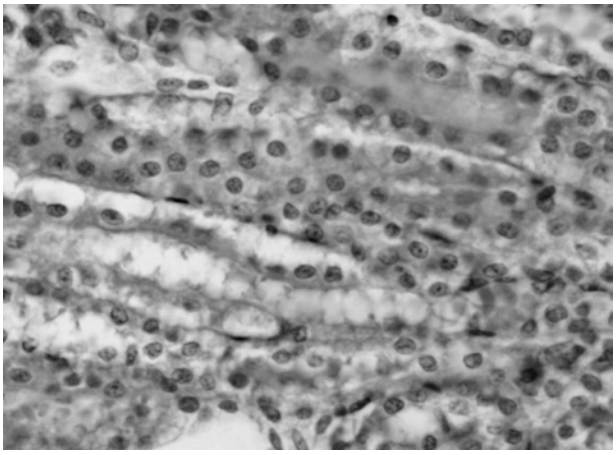

Figure 3. Kidney of guinea fowl (*Numida meleagris*) treated with 5.0 mg/kg feed/day of *Crotalaria retusa* seeds for 7 consecutive days, showing vacuolated renal tubular epithelial cells (HE, 100x).

Discussion

Natural cases of poisoning of birds by PA-containing plants have been reported in chickens (Hooper and Scanlan 1977, Pass et al. 1979, Alfonso et al. 1993, Gaul et al. 1994), ducks (Pass et al. 1979), and geese (Alfonso et al. 1993). The gross examination of chickens experimentally fed *C. spectabilis* seeds for 28 days revealed ascites and cachexia with liver volume increased or reduced with fibrin or subcapsule hematomas. The reported microscopic lesions of PA poisoning in birds include fatty degeneration, congestion, hemorrhage, and necrosis and megalocytosis of hepatocytes (Hatayde et al. 2008). The variations of observed lesions in the present study with those observed in chicken could be attributed to differences on administration period. In fact, the observed lesions in guinea fowl were

very similar to acute poisoning of sheep by *C. retusa* (Nobre et al. 2005).

Ascites is a common finding in PA poisoning, and was attributed to impaired serum protein synthesis in the liver and portal hypertension caused by damaged liver (Petterson and Culvenor 1983). However, both mechanisms should be excluded in affected guinea fowl in the present work because there was no hypoalbuminemia and no cirrhosis. From the several other known causes of ascites (McHutchison 1997, Hou and Sanyal 2009), the most plausible explanation for the cases in the present study is heart failure.

The evaluation of modes of cell death induced by monocrotaline in rats revealed that the hepatic parenchymal cells underwent coagulative oncosis in centrilobular regions and apoptosis in other regions (Copple et al. 2004). The observed centrilobular necrosis of hepatocytes in a guinea fowl from the present study was a possible result of that coagulative oncosis.

We found no significant difference between these treatment groups in any of the biochemical parameters that we analyzed. Since no biochemical evaluations were made in the guinea fowl that died earlier in the experiment, which could produce artifacts that were impossible to be identified or excluded, this assessment was made only in fowl that survived. The surviving guinea fowl could have become resistant to monocrotaline and other PAs present in *C. retusa*, and this resistance could have occurred through increased rate of hepatic conjugation of the toxin. In fact, it was observed that sheep treated with sub-lethal quantities of seeds of *C. retusa*, the same kind used in this study, became resistant (Anjos et al. 2010). However, this possible resistance should be further studied.

Among the various animal species, it is well known that there is a great variation in sensitivity to the toxic effects of PAs. Sheep, Japanese quail, rabbits, gerbils, hamsters, and guinea pigs are resistant to PA chronic toxicity, whereas chickens and turkeys are susceptible (Pierson et al. 1977, Cheeke and Pierson-Goeger 1983, Cheeke 1988, Cheeke and Huan 1988, Alfonso et al. 1993). The species variation in sensitivity is attributed to the need for bioactivation of these alkaloids to promote toxicity. The bioactivation occurs primarily through the cytochrome P450 enzyme complex, forming a highly reactive compound, the pyrrole, which combines with macromolecules such as proteins and DNA (Cheeke and Huan 1998, Huan et al. 1998, Kosogof et al. 2001). One form of variation in the sensitivity of animal species is the rate of bioactivation of PAs. In fact, Japanese quail (Buckmaster et al. 1977) and sheep (Huan et al. 1998) are highly resistant species because they have a low rate of pyrrole formation. Another feature of change is the detoxification of pyrrole compound that may occur by conjugation with glutathione or enzymatic degradation by hepatic esterases (Cheeke and Huan 1998, Huan et al. 1998, He et al. 2010). In sheep, the high efficiency in conjugation of pyrrole derivatives with GSH contributes to the resistance to PA poisoning (Swick et al. 1983, Huan et al. 1998).

In conclusion, guinea fowl (*N. meleagris*) are sensitive to the toxicity of PAs from *Crotalaria retusa*. Further, guinea fowl that survive the initial toxic insult may develop some resistance to PA intoxication.

References

Alfonso, H.A., L.M. Sanchez, M.A. Figueredo, and B.C. Gómez. 1993. Intoxication due to *Crotalaria retusa* and *C. spectabilis* in chickens and geese. *Veterinary and Human Toxicology* 35:539.

Anjos, B.L., V.M.T. Nobre, A.F.M. Dantas, et al. 2010. Poisoning of sheep by seeds of *Crotalaria retusa*: aquired resistance by continuous administration of low doses. *Toxicon* 55:28-32.

Buckmaster, G.W., P.R. Cheeke, G.H. Arscott, et al. 1977. Response of Japanese quail to dietary and injected pyrrolizidine (*Senecio*) alkaloid. *Journal of Animal Science* 45:1322-1325.

Cheeke, P.R. 1988. Toxicity and metabolism of pyrropizidine alkaloids. *Journal of Animal Science* 66:2343-2350.

Cheeke, P.R., and J. Huan. 1998. Species differences in bioactivation and detoxification of pyrrolizidine alkaloids. *In* T. Garland and A.C. Barr, eds., Toxic Plants and Other Natural Toxicants, pp. 559-563, CAB International, Wallingford, U.K.

Cheeke, P.R., and M.L. Pierson-Goeger. 1983. Toxicity of *Senecio jacobea* and pyrrolizidine alkaloids in various laboratory animals and avian species. *Toxicology Letters* 18:343-349.

Copple, B.L., C.M. Rondelli, J.F. Maddox, et al. 2004. Modes of cell death in rat liver after monocrotaline exposure. *Toxicological Sciences* 77:172-182.

Gaul, K.L., P.F. Gallagherm, D. Reyes, et al. 1994. Poisoning of pigs and poultry by stock feed contaminated with heliotrope seeds. *In* S.M. Colegate and P.R. Dorling, eds., Plant-Associated Toxins: Agricultural, Phytochemical and Economic Aspects, pp. 137-142, CAB International, Wallingford, U.K.

Hatayde, M.R., W.A.B. Pereira, G.S. Godoy, and A.C. Alessi. 2008. Efeitos da ingestão de sementes de *Crotalaria spectabilis* sobre o desempenho produtivo de galinhas poedeiras. *Veterinária Notícias* 14:19-28.

He, Y.Q., L. Yang, H.X. Liu, et al. 2010. Glucuronidation, a new metabolic pathway for pyrrolizidine alkaloids. *Chemical Research in Toxicology* 23:591-599.

Hooper, P.T., and W.A. Scanlan. 1977. *Crotalaria retusa* poisoning of pigs and poultry. *Australian Veterinary Journal* 53:109-114.

Hou, W., and A.J. Sanyal. 2009. Ascites: diagnosis and management. *The Medical Clinics of North America* 93:801-817.

Huan, J.Y., C.L. Miranda, D.R. Buhler, and P.R. Cheeke. 1998. Species differences in the hepatic microsomal enzyme metabolism of the pyrrolizidine alkaloids. *Toxicology Letters* 99:127-137.

Kosogof, C., J.J. Tepe, and R.M. Williams. 2001. DNA cross-linking by a photo-triggered pyrrolic progenitor developed from monocrotaline. *Tetrahedron Letters* 42:6641-6643.

McHutchison, J.G. 1997. Differential diagnosis of ascites. *Seminars in Liver Disease* 17: 191-202.

Nakage, A.P.M., M. Macari, L.S.O. Nakaghi, et al. 2000. Estudos hematológico e hormonal de frangos de corte tratados com contaminantes do milho: *Crotalaria spectabilis* e *Senna occidentalis*. *Brazilian Journal of Veterinary Research and Animal Science* 37:125-132.

Nobre, V.M.T., F. Riet-Correa, J.M. Barbosa Filho, et al. 2004. Intoxicação por *Crotalaria retusa* (Fabaceae) em eqüídeos no semiárido da Paraíba. *Pesquisa Veterinária Brasileira* 24:132-143.

Nobre, V.M.T., A.F.M. Dantas, F. Riet-Correa, et al. 2005. Acute intoxication by *Crotalaria retusa* in sheep. *Toxicon* 45:347-352.

Pass, D.A., G.G. Hogg, R.G. Russell, et al. 1979. Poisoning of chickens and ducks by pyrrolizidine alkaloids of *Heliotropium*. *Australian Veterinary Journal* 55:283-288.

Petterson, J.E., and C.C.J. Culvenor. 1983. Hepatotoxic pyrrolizidine alkaloids. *In* R.F. Keeler and A.T. Tu, eds., Handbook of Natural Toxins, pp. 637-671, Marcel Dekker, New York, NY.

Pierson, M.L., P.R. Cheeke, and E.O. Dickinson. 1977. Resistance of the rabbit to dietary pyrrolizidine (*Senecio*) alkaloid. *Research Communications in Chemical Pathology and Pharmacology* 16:561-564.

Swick, R.A., C.L. Miranda, P.R. Cheeke, and D.R. Buhler. 1983. Effect of phenobarbital on toxicity of pyrrolizidine (*Senecio*) alkaloids in sheep. *Journal of Animal Science* 56:887-894.

Thoden, T.C., and M. Boppré. 2010. Plants producing pyrrolizidine alkaloids: Sustainable tools for nematode management? *Nematology* 12:1-24.

Williams, M.C., and R.J. Molyneux. 1987. Occurrence, concentration, and toxicity of pyrrolizidine alkaloids in *Crotalaria* seeds. *Weed Science* 35:479-481.

Submitted: 9/17/2010
Accepted: 3/29/2011