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Effects of milk from goat fed *Crotalaria spectabilis* seeds on growing rats

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Efeitos da administração de ração contendo leite de cabra alimentada com sementes de *Crotalaria spectabilis* a ratos em crescimento

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SUMMARY

Seeds of *Crotalaria spectabilis*, containing the pyrrolizidine alkaloid (PA) monocrotaline (MCT), were fed to a lactating dairy goat. Milk from this goat was fed to rats for 8 weeks to determine whether MCT or its toxic metabolites are transferred into the goat's milk. Rats from the experimental group showed significantly higher ($p<0.05$) serum levels of ALT, AST, GGT and LDH and less weight gains ($p<0.05$) than control rats. The most significant lesions in rats consuming the experimental ration were mild to moderate interstitial pneumonia and a vacuolar degeneration and occasionally necrosis of periportal hepatocytes. The results of this study indicate that the PA and/or its metabolites are eliminated in milk.

UNITERMS: Milk; Pyrrolizidine alkaloids; *Crotalaria spectabilis*; Poisoning.

INTRODUCTION

Monocrotaline (MCT) is a pyrrolizidine alkaloid (PA) occurring in the seeds and aerial parts of many species of the legume *Crotalaria*. Representative species of this genus may be found throughout the world, distributed mainly in tropical and subtropical areas. *Crotalaria spectabilis* and other species have been introduced in many countries for use as soil builders and green manure[16].

It is well known that MCT is a pneumotoxic, hepatotoxic and nephrotoxic agent[1,6,9] which causes natural poisoning in almost all livestock[15,2,9] and man[9,12].

Although the primary concern of imposed toxicity is the consumption of *Crotalaria* seeds, PAs or their metabolites have been shown to pass into the milk of lactating animals, with potential human health implications[13,11]. Goats are of special interest because the high resistance to PA toxicity when compared with other species of domestic animals[6]. Thus, they can consume larger quantities of *Crotalaria* without manifesting clinical signs of intoxication; moreover, goats are usually raised on small farms in marginal agricultural areas that could be severely infested with *Crotalaria*. The purpose of the present study was to determine the possible toxic effects on growing rats of ration supplemented with milk from a dairy goat receiving *Crotalaria spectabilis* seeds.

MATERIAL AND METHOD

Crotalaria spectabilis

Seed of *C. spectabilis* were obtained from the Empresa de Pesquisa Agropecuária de Santa Catarina (EMPASC), Urussunga, State of Santa Catarina, Brazil.

Animals and experimental design

A 2-year old lactating dairy goat was used. The animal was kept under appropriate housing and acclimatized to the Research Center of Veterinary Toxicology (CEPTOX) for 10

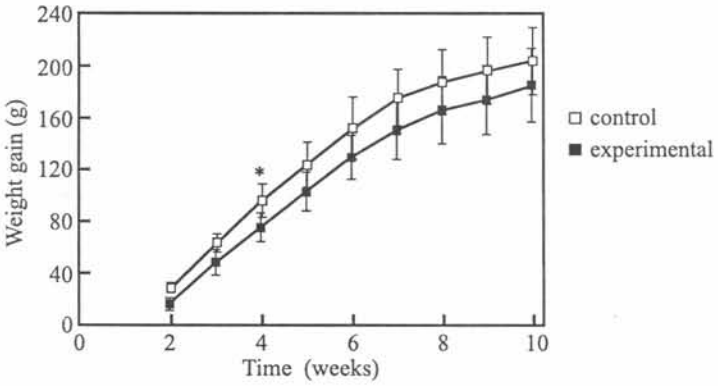

Figure 1

Weekly weight gains (mean and SEM) of rats fed ration containing milk from goat fed *Crotalaria spectabilis* seeds. * Significantly different from control at p<0.05% Multivariate Statistical Methods.

Table 1

Composition of the rat diets. Pirassununga - São Paulo, Brazil, 1994.

Ingredient	%
dried goat milk[a]	11.23
corn meal	36.51
soybean meal	21.55
meat and bone meal	11.43
wheat meal	13.18
kaolin	1.75
oil	0.87
salt	0.43
dicalcium phosphate	0.35
cassava flour	1.75
poultry premix	0.13
mineral premix	0.16
vitamin premix	0.66

[a]control rats received control milk (diet A) and experimental rats received experimental milk (diet B).

days prior the experiment.

For 2 weeks the goat was fed a commercial ration at rate of 2 kg/day. During another 2 weeks the commercial ration was supplemented with 0.5% of *C. spectabilis* seeds. Forage (*Brachiaria brizantha*) and water were available *ad libitum* throughout the experiment. Samples of milk collected from the first and the last two weeks were designated control (CM) and experimental milk (EM), respectively. Milk was collected twice daily, freeze dried and incorporated into a rat diet at proportion of 11.23% (Tab. 1).

Twenty male Wistar rats aged 22 days were randomly divided into 2 equal groups. The treatment imposed from the 22nd to 82nd day of age was diet A (control group) or diet B (experimental group). During the feeding period, weekly body weights were recorded. On the 83rd day, all animals were anesthetized and blood samples from a hepatic vein were taken for determination of serum alanine aminotransferase (ALT); aspartate aminotransferase (AST); alkaline phosphatase; albumin; creatinine; total protein; lactate dehydrogenase (LDH); gamma glutamyltransferase (GGT); urea; conjugated bilirubin and unconjugated bilirubin. The serum was assayed using a commercially available kit[a]. Immediately after blood

Table 2

ALT, AST, albumin, alkaline phosphatase, GGT, LDH, total protein, conjugated bilirubin, unconjugated bilirubin, creatinine and urea concentrations in sera from rats fed ration containing milk from a goat fed *Crotalaria spectabilis* seeds for 8 weeks. Data are reported as means ± SEM. Pirassununga - São Paulo, Brazil, 1994.

	Groups	
	Control	Experimental
ALT (U/L)	13.70 ± 2.31	15.80 ± 1.31 *
AST (U/L)	24.70 ± 3.40	30.50 ± 5.40 *
albumin (g/dl)	3.94 ± 0.42	4.05 ± 0.34
alkaline phosphatase (mU/ml)	338.5 ± 123.68	280.70 ± 56.79
GGT (UI/L)	12.13 ± 4.06	26.48 ± 6.29 *
LDH (U/L)	340.00 ± 92.61	445.50 ± 127.57*
conjugated bilirubin (mg/dl)	0.24 ± 0.14	0.23 ± 0.17
unconjugated bilirubin (mg/dl)	0.41 ± 0.22	0.43 ± 0.17
total protein (g/dl)	5.26 ± 0.72	5.30 ± 0.52
creatinine (mg/dl)	0.97 ± 0.10	1.00 ± 0.16
urea (mg/dl)	39.69 ± 5.12	44.10 ± 4.97

[a] Were used 10 animal per group.
* $p < 0.05$ Student T test.

[a]Reatoclin - CELM

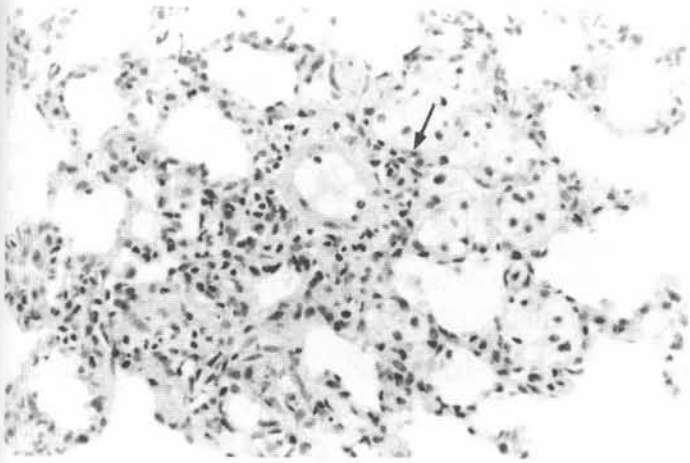

Figure 2
Photomicrography from the lung showing chronic interstitial pneumonia of a rat treated with diet containing milk from a goat fed *C. spectabilis* seeds. The alveolar septa are diffusely thickened by accumulation of fibrous tissue and lymphoid cells. Intra-alveolar macrophages are also observed (arrow). HE. 166x.

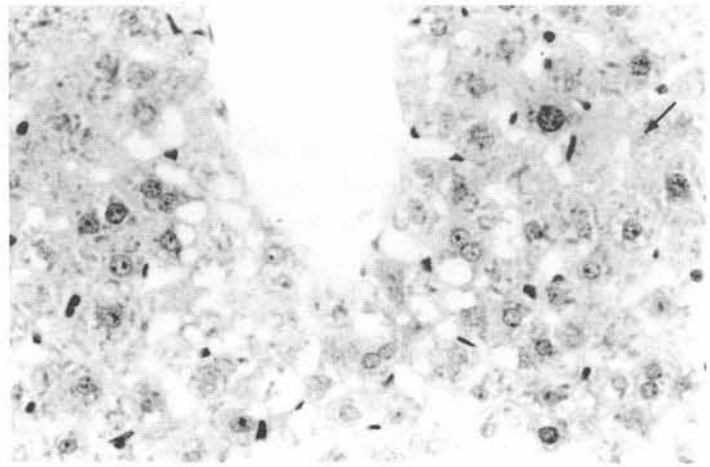

Figure 3
Photomicrography from the hepatic tissue of rat treated with diet containing milk from a goat fed *C. spectabilis* seeds. Note hepatocytes around the terminal hepatic vein showing vacuolar degeneration; among these cells there are hepatocytes presenting cariolysis (arrow) and others apoptosis. HE. 166x.

collection, the rats were euthanatized. Lung, liver and kidney tissues were collected, fixed in Bouin's liquid, embedded in parafin, sectioned at 5 μm and stained with hematoxylin and eosin for histopathologic examination.

Data were expressed as mean values and standard error of the mean and were analysed statistically by the Multivariate Statistical Methods[10], with the level of significance set at $p<0.05$.

Physical examination of the goat, to evaluate clinical status was performed weekly throughout the experiment and up to 2 months after the 1st day of *C. spectabilis* administration.

RESULTS

The effects of goat milk on the weight gain are shown in Fig. 1. The weight gain of experimental rats was significantly decreased ($p<0.05$) by the 3rd week of the experiment; however, from the 4th week to the end of the experiment, no differences were observed in this parameter, between control and experimental rats.

The biochemical data for the rats are presented in Tab. 2. Albumin, alkaline phosphatase, total protein, conjugated bilirubin, unconjugated bilirubin, creatinine and urea levels from animals treated with diet B did not differ from those of the control animals. On the other hand, rats from the experimental group showed significantly higher ($p<0.05$) serum levels of ALT, AST, GGT and LDH.

Main microscopic lesions in rats ingesting diet B were in the lungs. There was a mild to moderate interstitial pneumonia characterized by diffuse thickening of the alveolar septa by connective tissue and lymphoid cells (Fig. 2). Intra-alveolar macrophages (Fig. 2) and areas of emphysema were also observed. Other lesions observed in rats ingesting diet B were a vacuolar degeneration and, occasionally, cariolysis or apoptosis of periportal hepatocytes (Fig. 3). Vacuolar degeneration was also observed occasionally in the epithelium of renal tubules.

Crotalaria spectabilis seeds had no toxic effects on the goat during the study period.

DISCUSSION AND CONCLUSIONS

The interstitial pneumonia and the less weight gains observed in rats fed with diet B, strongly suggest that MCT and/or its metabolites were transferred to the milk goat consuming *Crotalaria spectabilis* seeds. Degeneration and necrosis of hepatocytes and increased activities of ALT, AST, GGT and LDH indicate the hepatotoxicity of diet B to rats. This diet essentially showed the same effects or those observed in domestic animals consuming Crotalaria[8].

There are wide variations among animal species in their susceptibility to PAs toxicity. Sheep and goats are highly resistant, whereas rats and mice are intermediate in susceptibility between goats and cattle[4,14,3]. These differences among animal species are associated to microsomal enzyme levels in the liver, and there are many evidences that the greater the resistance of animal to PA toxicity, the greater the danger that the alkaloids may be transferred to the milk[11]. Johnson[7] observed no toxicity in rats gavaged daily for 30 days with

milk from cows which died with severe signs of PA toxicity by *Senecio jacobea*. On the other hand, Goeger *et al.*[5] verified hepatic lesions in rats receiving the milk from a goat fed *Senecio jacobea*.

In conclusion, the results of the present experiment indicate that MCT or its metabolites were present in goat milk and were able to cause damage to the lungs, liver and kidney of rats. Thus, the toxicity of MCT via milk should be considered a risk for the health of the neonate and also for human consumption.

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RESUMO

Sementes de *Crotalaria spectabilis*, as quais contêm o alcalóide pirrolizidínico (AP), monocrotalina (MCT), foram moídas e administradas junto com a ração a uma cabra em lactação. O leite dessa cabra foi liofilizado e oferecido na ração por 8 semanas a ratos para determinar se a MCT ou seus metabólitos poderiam estar sendo significantemente eliminados no leite. Os ratos provenientes do grupo experimental apresentaram aumento significante ($p<0,05$) nos níveis séricos de ALT, AST, GGT e LDH e menor ganho de peso ($p<0,05$) que os ratos do grupo controle. As lesões mais significativas observadas nos animais que ingeriram a ração experimental foram pneumonia intersticial moderada e degeneração vacuolar e ocasionalmente necrose dos hepatócitos de região periportal. Os resultados deste estudo indicam que o AP e/ou seus metabólitos podem ser eliminados no leite.

UNITERMOS: Leite; Alcalóides de pirrolizidina; *Crotalaria spectabilis*; Intoxicação.

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