FULL PAPER *Toxicology*

Identification of a New Locoweed (*Oxytropis serioopetala*) and Its Clinical and Pathological Features in Poisoned Rabbits

Qin-Fan LI[1)], Cai-Ju HAO[1)], Yong-Ping XU[1)]*, Jie LIANG[1)], Kai YANG[1)] and Zhong-Hua CUI[1)]

[1)]*College of Veterinary Medicine, Northwest A&F Agriculture University, Yangling, Shaanxi 712100, China*

(Received 13 December 2011/Accepted 12 March 2012/Published online in J-STAGE 26 March 2012)

ABSTRACT. By a series of experiments, we identified a new member of the locoweed family, *Oxytropis serioopetala*, that produces swainsonine, a phytotoxin harmful to livestock. In order to evaluate the toxicity of *Oxytropis serioopetala*, its extract was administered to ten rabbits by gavage at a dose of 1.5 mg/kg body weight as swainsonine once daily. After the 20th day, the rabbits appeared depressive and anorexic. In addition, intention tremors were apparent upon movement. Their eyes were dull. The rear limbs were severely weak and even progressed to partial paresis. The activities of serum aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (AKP) and urea nitrogen (BUN) levels in the poisoned rabbits increased significantly. Serum α-mannosidase (AMA) activity decreased markedly. Pathomorphological lesions in the locoweed-poisoned rabbits developed severe microvacuolation of visceral and neurological tissue. Extensive vacuolation was observed in the liver, kidney and brain. These clinical and pathological features are similar to the symptoms of locoism.

KEY WORDS: locoweed, *Oxytropis serioopetal*a, pathology, swainsonine.

doi: 10.1292/jvms.11-0557; *J. Vet. Med. Sci.* 74(8): 989–993, 2012

Locoweeds are perennial herbaceous plants of the *Astragalus* spp. and *Oxytropis* spp. containing the toxic indolizidine alkaloid swainsonine [9]. The structure of this compound is shown in Fig. 1. Consumption of locoweed results in a toxicity syndrome known as locoism, which is characterized by neurological changes, reproductive disturbance, emaciation and eventually death [13]. Reported histologic lesions include widespread neuronal vacuolation and axonal dystrophy as well as vacuolation of the kidney, liver and various endocrine tissues [7, 15, 18, 19, 21].

Locoweed poisoning is the most widespread poisonous plant problem in many countries including China [7], United States [14] and Canada [5]. There are 270 species of *Astragalus* and 120 species of *Oxytropis* in China. Ten species of *Astragalus* and *Oxytropis* are reported as locoweeds and widely grow in the western rangelands [15]. In recent years, locoweeds have covered up to 11 million hectares. At the same time, some new species are found to be dominant species [23]. *Oxytropis serioopetala* is one of the most important poisonous plants and mainly grows on the sand beach in south Tibet, P.R. China. Domestic animals may be poisoned, even die, after grazing on this. *Oxytropis serioopetala* poisoning has caused a large economical loss in the region [22]. However, there is no detailed report about the toxicity of *Oxytropis serioopetala*. Therefore, the purpose of this study was to identify the toxic principles of *Oxytropis serioopetala* and describe the clinical signs and histological lesions of chronic *Oxytropis Serioopetala* poisoning in rabbits.

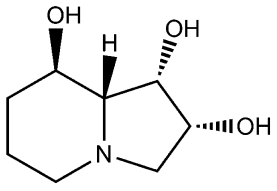

Fig. 1. Structure of swainsonine [(1S,2R,8R,8aR)-1,2,8-trihydroxyoctahydroindolizine, mol wt. 173.1].

MATERIALS AND METHODS

Plant sample preparation: *Oxytropis serioopetala* (identified by Dr. Yuping Li, a botany professor at College of Life Science, Northwest A&F University, Yangling, Shaanxi, P.R. China) was collected from Qushui, Tibet, P.R. China (GPS coordinates 290.35.37'N 900.57.29' E). The plants were air-dried, ground and passed through a 1-mm mesh. Then, the ground plants were mixed, and random samples were collected for isolation and extraction of swainsonine.

Isolation and identification of swainsonine: Swainsonine was isolated from Oxytropis serioopetala in our laboratory following the protocol reported previously [2, 4, 8]. The identification of swainsonine was performed by melting point measurement, gas chromatography (GC) and mass spectrometric (MS) [9, 11].

Preparation of Oxytropis serioopetala extract: The *Oxytropis serioopetala* extract was prepared as described

*CORRESPONDENCE TO: XU,Y.-P., College of Veterinary Medicine, Northwest A&F Agriculture University, Yangling, Shaanxi 712100, China.
e-mail: ypxu68@yahoo.com.cn

by Taylor and Strickland [20]. The ground *Oxytropis serioopetala* was boiled in methanol for 24 hr. The liquid fraction was filtered, and methanol was removed. The residue were vigorously mixed 1 to 9 with distilled water and centrifuged at 1,500 × *g* for 30 min. The final water-soluble fraction of the extract contained 4.51 mg/m*l* swainsonine, according to α-mannosidase inhibition assay [20]. The extract was used for subsequent animal experiments as described below.

Animals and grouping: A total of 20 adult New Zealand white rabbits were purchased from the Laboratory Animal Center of Northwest A&F University (Yangling, Shaanxi, P.R. China). Body weight ranged from 1.68 to 2.19 kg. All experiments conducted with these animals were based on the institutional and ethical guidelines involving use of animals (The Committee of Science and Technology of the People's Republic of China, 1988).

Poisoning experiment with Oxytropis serioopetala extract: Rabbits were randomly assigned into a treated group (n=10) and control group (n=10). The treated group received *Oxytropis serioopetala* extract dissolved in water by gastric tube at a dose of 1.5 mg/kg body weight of swainsonine once daily. The exposure to *Oxytropis serioopetala* extracts at the same frequency continued for 60 days to develop locoism. The control group received only swainsonine-free water. During experiments, the animals were inspected for psychosis, appetite, clinical signs of swainsonine intoxication and especially intention tremors, weakness and paresis of limbs, etc. each morning.

Blood sample collection and serum preparation: Blood samples (5 m*l*) were collected from the small saphenous vein on day 0, 10, 20, 30, 40, 50 and 60, and the serum of each rabbit separated.

Analysis of serum swainsonine, enzymes and urea nitrogen: Serum swainsonine, α-mannosidase (AMA), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (AKP) and urea nitrogen (BUN) were assayed following the protocol described previously [8, 19, 20]. All measurements were performed on an UV-VIS spectrophotometer (SPECORD 50 PC, Analytik Jena AG) at room temperature using a 1 cm path length cuvette.

Pathological evaluation: Rabbits were humanely euthanized with sodium pentobarbital (0.5 mg/kg IV) and necropsied on day 60 of the study. Cerebellums, cerebrums, livers, kidneys, lungs, spleens and hearts were collected. Tissues were fixed with 4% paraformaldehyde in 0.1 M PBS and then embedding of paraffin. The 4-μm-thin sections were stained with hematoxylin and eosin (HE). Histological examination of tissue was performed by conventional microscopy.

Statistical analysis: The significance of differences between groups was calculated using Student's *t*-test or one-way ANOVA analysis as appropriate. Statistical analyses were performed with SPSS software version 13.0 (SPSS Inc. Chicago, IL, U.S.A.) and two-tailed $P<0.05$ was considered statistically significant.

RESULTS

Melting point measurement, gas chromatography (GC) and mass spectrometric (MS): The crystals isolated from *Oxytropis serioopetala* were subjected to melting point measurement and GC and MS assays. The results showed that the melting point and GC retention time were 144–145°C and 1.67 min, respectively. The MS fragmentation pattern was identical to those of swainsonine according to EI-MS m/e: 173 (M+), 155 (M-H_2O), 138 (M-H_2O-OH), 116 (M-2H_2O-OH), 115, 96, 84, 72 and 43 (Fig. 2). These data were consistent with previous reports about swainsonine [1, 8]. The results indicated distinctly that swainsonine, the phytotoxin of locoweed, is the principle toxin in *Oxytropis serioopetala.*

Appearance and behavioral changes: After 20 days of continuous exposure to *Oxytropis serioopetala* extract, all the rabbits appeared depressive and exhibited anorexia and gradual emaciation. In addition, intention tremors were apparent upon movement. Their eyes were dull. The hind limbs were severely weak and even progressed to partial paresis. These clinical signs were similar to those of locoweed-poisoned animals.

Analysis of serum swainsonine and AMA: The total swainsonine level increased with the exposure time, as shown in Fig. 3A. The serum swainsonine level of the treated group continuously increased over the entire experimental period (day 1 to 60), and reached a maximal level on day 60 (Fig. 3A). No swainsonine was detected in the serum from control group animals. At the same time, The serum AMA activity of the treated group declined significantly from the beginning of the experiment. During the entire experiment, the serum AMA activity of the control group was much higher than of the treated group ($P<0.01$) (Fig. 3B).

The exposure to *Oxytropis serioopetala* extract steadily elevated serum ALT levels in the treated group, and the maximum level was achieved on day 60 (Fig. 4A). Compared with the control group, the serum ALT levels in the treated group were significantly higher from day 20 to 60 ($P<0.01$). Similar to the change in serum ALT, the serum levels of AST, AKP and BUN in the treated group were much higher than those of the control group from day 20 to 60 ($P<0.01$) (Fig. 4B, 4C and 4D).

Pathological: Postmortem examination showed that the liver was slightly enlarged and that all the lymph nodes were swollen and edematous in the treated group. Excessive serous fluid (0.05 to 0.1 *l*) was observed in the peritoneal cavity of 5 rabbits from the treated group. No other gross lesions were found.

Histologically, no significant vacuolation was observed in organs from the control rabbits (Fig. 5a, 5b, 5c and 5d). All of the *Oxytropis serioopetala*-poisoned rabbits developed severe microvacuolation of visceral and neurological tissue. Neurons throughout the central nervous system, including most ganglia, were also vacuolated. The neuronal vacuolation was especially prominent in cerebellar Purkinje cells (Fig. 5A). Some cerebrum neurons may be lost in severely poisoned rabbits. The vacuolation expanded the cytoplasm, with little tendency to displace or shrunken compress the nucleus (Fig. 5B). Similar pathologic changes were also found in hepatocytes (Fig.5C), the renal convoluted tubular epithelia (Fig. 5D), bronchial epithelial cells, pneumocytes

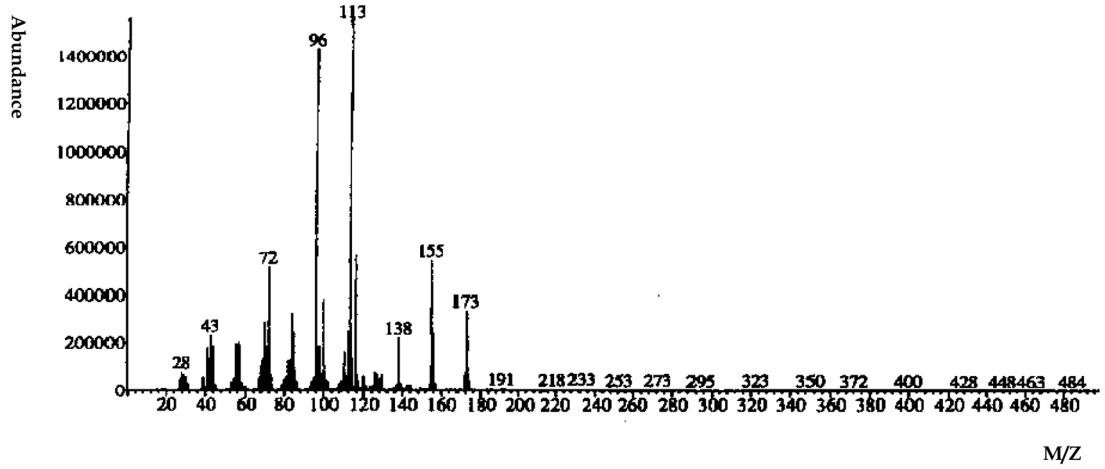

Fig. 2. Mass spectrum of white crystals obtained by mass spectrometric. The numbers marked, 173 (M+), 155 (M-H_2O), 138 (M-H_2O-OH), 116 (M-2H_2O-OH), 115, 96, 84, 72 and 43, are all mother nucleus pyrolysis peaks. These peaks were consistent with previous reports concerning swainsonine.

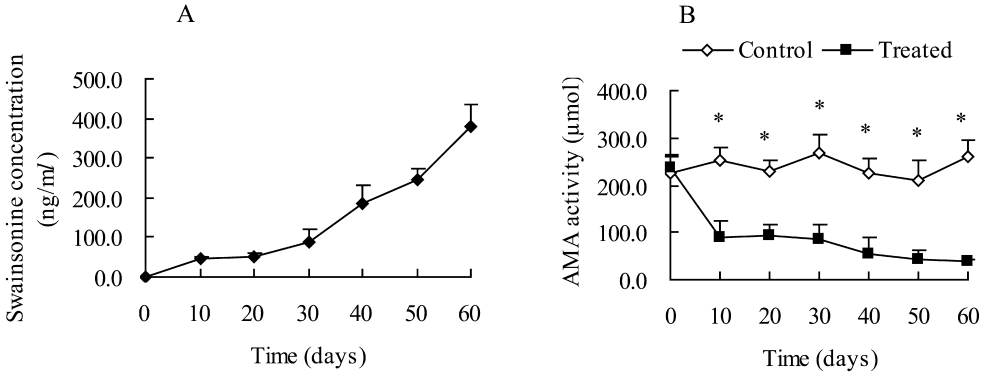

Fig. 3. Alteration of serum swainsonine concentration and AMA activity. The serum level of swainsonine (A) and serum AMA activity (B) in the *Oxytropis serioopetala* extract treated group from day 0 to 60. * Indicates a statistically significant difference (P<0.01) between the control and treated groups.

and various cells of the monocyte macrophage system.

DISCUSSION

The principle toxin in locoweed has been identified as swainsonine, an indolizidine alkaloid [10], which inhibits α-mannosidase and thereby results in oligosaccharide accumulation in lysosomes and pathological lesions that are similar to heritable mannosidosis, a lysosomal storage disease [3]. Therefore, serum α-mannosidase is considered a marker of locoweed intoxication. The inhibition of α-mannosidase activity strongly correlates with swainsonine dose [19]. In the present study, swainsonine isolated from *Oxytropis serioopetala* was identified. The serum AMA activity of the treated group, which was exposed to the extract from *Oxytropis serioopetala*, declined significantly (P<0.01). At the same time, the serum swainsonine level of the treated group continuously increased. These results suggest that *Oxytropis serioopetala* is a new species of locoweed and that serum α-mannosidase activity may be useful in documenting exposure to locoweeds and monitoring intoxication [19].

Although the toxicity of the plant and its effects on animals have been reported, the detailed clinical signs and pathological changes still remain unknown (Yu *et al.*, 2006). The symptoms, including depression and slight intention tremors and partial paresis caused by exposure to *Oxytropis serioopetala* extract in rabbits, are quite similar to the clinical course in large animals [7, 19, 23]. Previous reports showed that cattle and sheep are most often lethargic and exhibit muscular weakness, intention tremors, proprioceptive deficits and obvious behavioral changes [6, 13, 16]. Horses are also reluctant to move and often docile to very nervous and excitable [17].

AST, ALT, AKP and BUN are believed to be good indicators for diagnosis of locoweed poisoning in animals [12, 19]. The increase in serum AST, ALT and AKP activity has been associated with cellular damage including lesions in the brain, liver, skeletal muscle, heart and other tissues. The in-

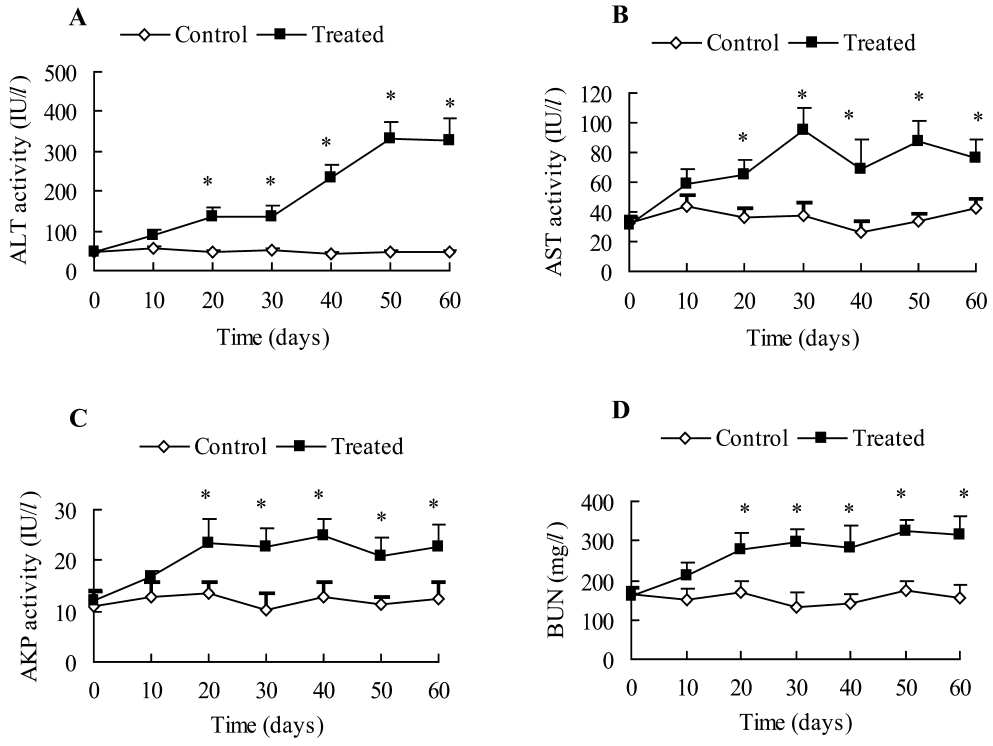

Fig. 4. Changes of blood biochemical values and BUN level caused by *Oxytropis serioopetala* exposure. Increases in the activity of ALT (A), AST (B) and AKP (C) and level of BUN (D) in serum of rabbits exposed to *Oxytropis serioopetala* extract from day 0 to 60. * Indicates a significant difference ($P<0.01$) between the control and treated groups.

crease in serum BUN level is relative to the cellular damage of kidney [12, 18]. In this work, the increases in serum AST, ALT, AKP and BUN in poisoned rabbits were consistent with these reports. These blood biochemical indexes dovetail nicely with pathological alterations described above.

All the changes in this study are similar to those of locoism caused by grazing on locoweeds in livestock [12, 19]. Swainsonine inhibits α-mannosidase and thereby results in oligosaccharide accumulation in lysosomes, and the resulting in vacuolation in liver, kidney and brain cells confirms the biochemical indexes [3]. In this study, the serum levels of ALT, AST, AKP and BUN in the treated group were much higher than those of the control group from day 20 to 60, which indicated that brain, liver, skeletal muscle and heart were damaged. The vacuolation of brain cells could explain the intention tremors and paresis, which we detected clinically.

In conclusion, this study demonstrated that *Oxytropis serioopetala* contains swainsonine, the toxic component of locoweed, by melting point measurement, gas chromatography and mass spectrometry. Further animal experiments in rabbits showed that both *Oxytropis serioopetala* poisoning and locoism have similar pathological and clinical features. It is suggested that *Oxytropis serioopetala* is one of the new locoweeds. Ruminants poisoned by *Oxytropis serioopetala,* It could be treated with the methods reported for locoweed.

REFERENCES

1. Colegate, S. M., Dorling, P. R. and Huxtable, C. R. 1979. A spectroscopic investigation of swainsonine: an α-mannosidase inhibitor isolated from *Swainsina canecens*. *Aust. J. Chem.* **32**: 2257–2264. [CrossRef]
2. Davis, D., Schwarz, P., Hernandez, T., Mitchell, M., Warnock, B. and Elbein, A. D. 1984. Isolation and characterization of swainsonine from Texas locoweed (*Astragalus emoryanus*). *Plant Physiol.* **76**: 972–975. [Medline] [CrossRef]
3. Dorling, P. R., Huxtable, C. R. and Colegate, S. M. 1980. Inhibition of lysosomal α-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*. *Biochem. J.* **191**: 649–651. [Medline]
4. Gardner, D. R., Lee, S. T., Molyneux, R. J. and Edgar, J. A. 2003. Preparative isolation of swainsonine from locoweed: extraction and purification procedures. *Phytochem. Anal.* **14**: 259–266. [Medline] [CrossRef]
5. Harries, W. N., Baker, F. P. and Johnston, A. 1972. Case report: an outbreak of locoweed poisoning in horses in southwestern Alberta. *Can. Vet. J.* **13**: 141–145. [Medline]
6. James, L. F. 1976. Effect of locoweed (*Astragalus lentiginosus*) feeding on fetal lamb development. *Can. J. Comp. Med.* **40**: 380–384. [Medline]
7. Li, J. K. 2003. The present situation and prospect of the studies on locoweed in China. *Sci. Agric. Sin* **36**: 1091–1099.
8. Li, Q. F., Wang, J. H., Li, R., Chen, Y. and Liu, Z. B. 2005. Detection of alkaloid and isolation of swainsonine from *Oxytropis glacialis*. *Acta. Vet. Zootech. Sin.* **36**: 1339–1343 (in Chinese).

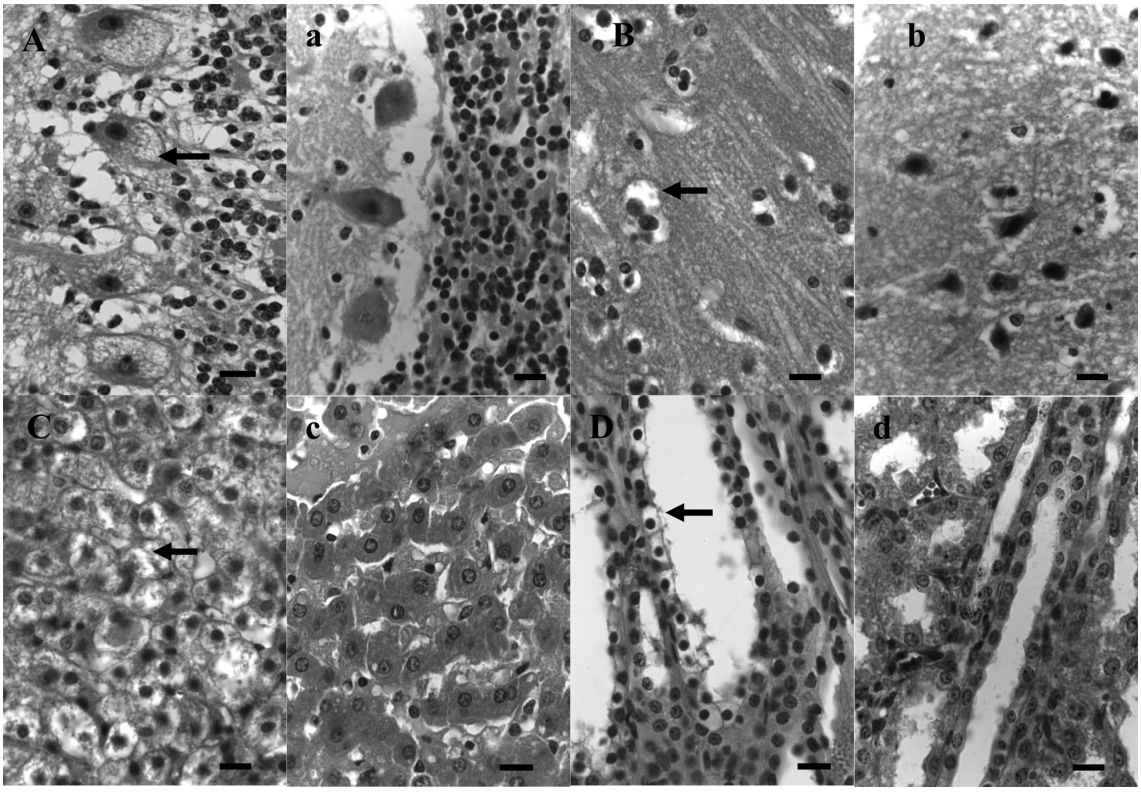

Fig. 5. Vacuolation in various tissues from rabbits exposed to *Oxytropis serioopetala* extract for 60 days. Vacuolation (indicated by arrows) was distributed in the cerebellum Purkinje cells (A), cerebrum neural cells (B), hepatocytes (C) and renal convoluted tubular epithelium (D) and can be contrasted with normal cells in photograph a, b, c and d, respectively. HE. Bar=15 μm.

9. Molyneux, R. J., Benson, M. and Wong, R. J. 1988. Australine, a novel pyrrolizidine alkaloid glucosidase inhibitor from *Castanospermum australe*. *J. Nat. Prod.* **51**: 1198–1206. [CrossRef]
10. Molyneux, R. J. and James, L. F. 1982. Loco in toxic at ion indolizidine alkaloids of spotted locoweed (*Astragalus lentiginosus*). *Science* **216**: 190–191. [Medline] [CrossRef]
11. Molyneux, R. J., McKenzie, R. A., O'Sullivan, B. M. and Elbein, A. D. 1995. Identification of glycosidase inhibitors swainsonine and calystegine B2 in Weir Vine (Ipomoea sp. Q6 [aff.calobra]) and correlation with toxicity. *J. Nat. Prod.* **58**: 878–886. [Medline] [CrossRef]
12. Pfister, J. A., Stegelmeier, B. L., Gardner, D. R. and James, L. F. 2003. Grazing of spotted locoweed (A*stragalus lentiginosus*) by cattle and horses in Arizona. *J. Anim. Sci.* **81**: 2285–2293. [Medline]
13. Plumlee, K. H. and Galey, F. D. 1994. Nuro toxicmyco toxins: a review of fungal toxins that cause neurological disease in large animals. *J. Vet. Intern. Med.* **8**: 49–54. [Medline] [CrossRef]
14. Ralphs, M, H. and James, L. F. 1999. Locoweed grazing. *J. Nat. Toxins* **8**:47–51.
15. Shi, Z. C. 1997. Major poisonous plants of China grassland, 1st ed. China agriculture press, Beijing (in Chinese).
16. Stegelmeier, B. L., James, L. F., Panter, K. E., Gardner, D. R., Pfister, J. A., Ralphs, M. H. and Molyneux, R. J. 1999. Dose response of sheep with locoweed poisoning. *J. Vet. Diagn. Invest.* **11**: 448–456. [Medline] [CrossRef]
17. Stegelmeier, B. L., James, L. F., Panter, K. E., Gardner, D. R., Ralphs, M. H. and Pfister, J. A. 1998. Tissue swainsonine clearance in sheep chronically poisoned with locoweed (*Oxytropis sericea*). *J. Anim. Sci.* **76**: 1140–1144. [Medline]
18. Stegelmeier, B. L., Molyneux, R. J., Elbein, A. D. and James, L. F. 1995. The lesions of locoweed (*Astragalus mollissimus*), swainsonine, and castanospermine in rats. *Vet. Pathol.* **32**: 289–298. [Medline] [CrossRef]
19. Stegelmeier, B. L., Ralphs, M. H., Gardner, D. R., Molyneux, R. J. and James, L. F. 1994. Serum α-mannosidase activity and the clinicopathologic alterations of locoweed (*Astragalus mollissimus*) intoxication in range cattle. *J. Vet. Diagn. Invest.* **6**: 473–479. [Medline] [CrossRef]
20. Taylor, J. B. and Strickland, J. R. 2002. Appearance and disappearance of swainsonine in serum and milk of lactating ruminants with nursing young following a single dose exposure to swainsonine (locoweed; *Oxytropis sericea*). *J. Anim. Sci.* **80**: 2476–2484. [Medline]
21. Tulsiani, D. R. P., Broquist, H. P. and Touster, O. 1985. Marked differences in the swainsonine inhibition of rat liver lysosomal alpha-D-mannosidase, rat liver Golgi mannosidase II, and jack bean alpha-D-mannosidase. *Arch. Biochem. Biophys.* **236**: 427–434. [Medline] [CrossRef]
22. Yu, Y. T., Wang, J. H., Fu, G. J., Wang, L. F. and Wang, Y. W. 2008. Nutrition contents and mineral elements in Pod-bearing period *Oxytropis serioopetala*. *Pratac. Science* **25**: 48–51 (in Chinese).
23. Zhao, B. Y., Tong, D. W., Ge, P. B., Dong, Q. and Mo, C. H. 2003. Locoweed harm investigation in the west grassland of China. *Grassl. China* **25**: 65–68 (in Chinese).