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Short communication

Effect of *Ageratina adenophora* on hepatic and pulmonic pathological lesions in horses

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Abstract

The effect of *Ageratina adenophora* on pathological characteristics of the liver and lungs as well as serum biochemical parameters in horses were investigated. Ten horses without ingestion history of *Ageratina adenophora* were classified into the control group, and 10 poisoned but survived horses with 3 months ingestion history were set as the case group. Results showed that serum AST, ALT, ALP, magnesium and phosphorus were elevated significantly, while creatinine was decreased remarkably. Hematoxylin and eosin staining of liver tissues showed diffuse swelling or destruction of hepatocytes, narrowing or atrophy of the hepatic sinusoids, and little lymphocytic infiltration; lung tissues presented destroyed alveoli and inflammatory cell infiltration.

Key words: *Ageratina adenophora*, liver, lung, horse, toxicity

Introduction

Ageratina adenophora (*A. adenophora*), belonging to genus *Eupatorium*, is a perennial semi-shrub in the family of *Asteraceae*. It was firstly reported in Yunnan province in the 1940s and spread rapidly in the southwest region of China during several decades, causing huge damages to both ecological environment and modern agricultural production. Previous studies revealed that livestock with intake of *A. adenophora* showed clinical signs such as diarrhea, moult, abortion and even death, and animals may present asthma (Jie et al. 2018). Specially, several cases of *A. adenophora* toxicity in horses were reported in Queensland with respiratory disease and lung lesions, but no significant histopathological changes were discovered in the liver and kidneys (O'Sullivan 1979, 1985). To date, there was no *A. adenophora* poisoning in horses reared on Yunnan-Guizhou plateau reported in China. High-altitude Yunnan-Guizhou Plateau is characterized by a series of parallel alpine ridges with dramatic ecological stratification. Herein, this study was performed to investigate the effects of *A. adenophora* on the liver, kidneys and lungs in horses according to the pathological changes and as well as on blood biochemical parameters.

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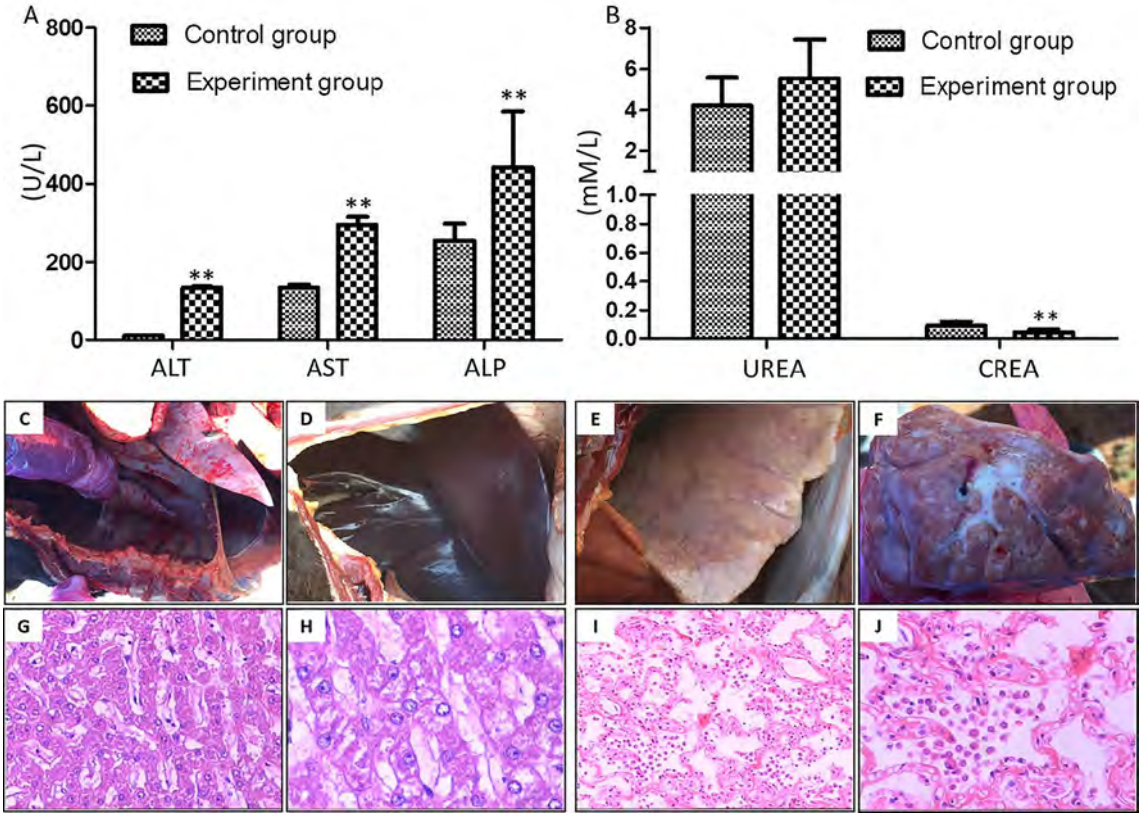

Fig.1. The effect of *A. adenophora* on liver, kidney and lung in horses. A) Liver function indexes. B) Kidney function indexes (mean±SD). C) D) E) F) The visual lesions of liver and lung. G) H) I) J) H&E staining of liver and lung tissues (200× and 400×). ** Significantly different from control group ($p<0.01$).

Materials and Methods

Twenty-one horses included in this study were from the Lingjun International Equestrian Club located in the Yunnan-Guizhou plateau of China. All animal experiments were performed with the approval of the Animal Care Committee of the Yunnan Agricultural University. Ten horses without ingestion history of *A. adenophora* were classified into the control group, and 10 poisoned but survived horses with 3 months ingestion history were set as the case group. All the animals had no contact history with other toxic substances except *A. adenophora* during the 3 months. The poisoned horses were diagnosed according to feeding history and clinical symptoms. Clinical symptoms were presented after horses were grazed on a mountain land with *A. adenophora* for more than 2 months. They manifested: sneezing, transparent nasal mucus, coughing, and developed into serous nasal mucus, congestion of eye conjunctiva. Severe cough was observed 4 days later. Dyspnoea and convulsions was observed after 7 days; ultimately one of the horses was dead due to dyspnoea. To investigate the liver and kidney function, 5 mL of blood samples were collected from the jugular vein for biochemical analysis [Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CRE), calcium, magnesium and phosphorus] with semi-automatic biochemistry analyzer (IDEXX Vet Test 8008, Maine, USA). The poisoned dead horse among the case group was necropsied and samples of the liver and lungs were carefully dissected out and fixed in 10 % neutral buffered formalin. Samples were stained with hematoxylin and eosin (HE) according to the Feldman and Wolfe protocol (Feldman and Wolfe 2014) for histological analysis. Images were taken under Olympus light microscope (BX53). Statistical analyses were performed using student's test by SPSS (version 21.0, SPSS Inc, USA). $p<0.01$ value was considered statistically significant.

Results and Discussion

As shown in Fig. 1, the parameters of the liver function including AST, ALT and ALP were significantly promoted in poisoned horses compared with those in the controls ($p<0.01$) (Fig. 1A). Previous studies indicated that when hepatic cells were damaged due to fibrogenesis, the ALP activities increased correspondingly. The levels of AST and ALT were significantly increased in case of pathology associated with

Table 1. The serum magnesium, phosphorus and calcium in poisoned horses.

Test items (M/L)	Reference value	Case group	Description
magnesium	0.66-0.95	2.6	increase
phosphorus	0.74-1.39	2.5	increase
calcium	2.72-3.22	2.9	normal

necrosis in the liver (Rhiouani et al. 2008). At necropsy, the thickness and adhesion of the pleura was increased (Fig. 1C). The liver appeared brown or dark chocolate color and it was a little evaginable on the cut surface (Fig. 1D). HE staining of liver tissues showed diffuse swelling or destruction of hepatocytes, narrowing or atrophy of hepatic sinusoids, and lymphocytic infiltration (Fig. 1G and H). Kidney function index of creatinine was declined significantly ($p<0.01$) while there was no significant change in BUN concentration (3.6-7.1 mM/L) (Fig. 1B), although it was increased when compared with that in the controls. In addition, serum magnesium and phosphorus were boosted in certain extent while the concentration of calcium was maintained in the normal range (Table 1).

Moreover, necropsy lesions in lungs included the pleural adhesion, and clear texture of pulmonary edema was observed (Fig. 1E). Pale serous exudate was also found on the lung surface and within the parenchyma (Fig. 1F). At microscopic examination, alveoli were destroyed and inflammatory cells infiltrated the alveolar space (Fig. 1I). Proliferation of Clara cells were presented in the alveolar septa (Fig. 1J). The lesions in lungs described above were similar to those reported in donkeys, with characteristic of Clara cells proliferation and interstitial fibrosis (Pessoa et al. 2013). It was reported that *A. adenophora* impaired spleen function in mice through oxidative stress damage and pyroptosis, (Sun et al. 2019). Taken together, elevated serum AST, ALT and ALP activities, and lesions found in the liver and lungs imply that *A. adenophora* elicited hepatotoxicity and lung injuries in horses reared on the Yunnan-Guizhou plateau of China.

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