HEPATOTOXIC EFFECT OF *EUPATORIUM INULIFOLIUM* IN THE RAT

SJAMSUL BAHRI, D.R. STOLTZ and DIAH PARAMARDINI*
Balai Penelitian Veteriner, Bogor
**Fakultas Kedokteran Hewan – IPB, Bogor*
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ABSTRACT

A study on the toxicity of *Eupatorium inulifolium* in the rat was conducted to determine if this plant might be toxic to livestock. Eighteen young rats were used in three treatment groups of six animals each. The treatments were: (1) control diet, (2) 12.5% *Eupatorium* in diet, and (3) 25% *Eupatorium* in diet. The treatments were continued for three weeks. Histological examination of livers of rats fed 12.5% and 25% *Eupatorium* revealed megalocytosis and individual necrosis of hepatocytes. Mild to moderate bile duct proliferations were also observed. No lesions were seen in other organs. It was concluded that *Eupatorium inulifolium* is a hepatotoxic plant.

ABSTRAK

Eupatorium inulifolium merupakan salah satu dari enam tanaman yang dicurigai menjadi penyebab kematian ternak sapi di Sumatera Utara, tetapi efek toksik dari tanaman ini belum diketahui. Dalam tulisan ini dilaporkan suatu percobaan mengenai efek toksik tanaman *E. inulifolium* pada tikus putih. Dalam penelitian ini digunakan 18 ekor tikus muda yang dibagi menjadi tiga kelompok perlakuan, masing-masing dengan 6 ekor tikus sebagai ulangan. Perlakuan terdiri atas: (1) pakan tanpa *Eupatorium* (kontrol); (2) pakan dengan 12,5% *Eupatorium*; dan (3) pakan dengan 25% *Eupatorium*. Perlakuan diberikan setiap hari selama 3 minggu. Pada pemeriksaan secara histologik organ hati dari tikus-tikus yang diberi 12,5% dan 25% *Eupatorium* terlihat adanya megalositosis dan nekrosis dari beberapa sel-sel hati. Perubahan berupa proliferasi dari saluran-saluran empedu juga dapat diamati pada percobaan ini. Pada organ-organ lain tidak didapatkan adanya perubahan. Dari hasil penelitian ini dapat dinyatakan bahwa *Eupatorium inulifolium* merupakan suatu tanaman yang bersifat hepatotoksik.

INTRODUCTION

In Indonesia there are many plants which have been found responsible for serious disease and mortality of livestock in other parts of the world. Nevertheless, very little is known about the effects of poisonous plants on livestock production and health in Indonesia.

Liver cirrhosis was reported in 58 of 250 dairy cattle that had died of suspected plant poisoning in North Sumatra in early 1984. Investigation of this case suggested that six plants be considered high priority for further study of hepatotoxicity (Murdiati and Stoltz, 1987). One of these plants is *Eupatorium inulifolium*, a common weed in Indonesia and presents the subject of this report.

The genus *Eupatorium* belongs to the family Compositae (Asteraceae). The majority of *Eupatorium* species are found in the tropical parts of America, of which three have been reported toxic to livestock.

Eupatorium rugosum, commonly known as white snakeroot in North America, causes a disease called milk sickness in people consuming milk from cows that have grazed the plant (Cheeke and Shull, 1985). In cattle, sheep, goats, horses, pigs and birds, white snakeroot produces a disease known as "trembles" (Everist, 1981). The toxic component is not yet identified (Beier *et al.*, 1987).

Eupatorium adenophorum or crofton weed and *E. riparium* or mist flower are both known to cause respiratory disease in horses in Australia and Hawaii (O'Sullivan, 1979; Gibson and O'Sullivan, 1984; O'Sullivan, 1985; O'Sullivan *et al.*, 1985), suggesting that they contain similar but as yet unidentified toxins.

Eupatorium inulifolium and *E. odoratum* are common in Indonesia. *E. inulifolium* generally grows in low and high areas in Sumatra and Java (Koster, 1935). The local name of *E. inulifolium* in North Sumatra is "si bentar bunga" and in Java the plant is known as "babanjaran", "kipapatong" or "kirinyuh" (Steenis, 1978). *E. inulifolium* grows in the late dry season, reaching 1-5 m in height with a white composite-type flower. Normally animals do not eat this plant, but during the dry season when other forage becomes scarce, *Eupatorium* stays green and livestock are forced to graze on this plant.

The purpose of the study reported here is to determine the hepatotoxic effect of *Eupatorium inulifolium* in rats as a follow up to the previous investigation of suspected plant poisoning of North Sumatran cattle (Murdiati and Stoltz, 1987).

MATERIALS AND METHODS

Plant material was collected from the field at Rancamaya, Bogor, and dried at 50°C in an oven. Leaves were separated and milled to a fine powder. Their identity was confirmed by staff of the Bogor Herbarium. The dried powdered leaf was incorporated into the diet at 12.5 and 25%. The standard rat diet with no *Eupatorium* served as control. All diets were in pelleted form.

Eighteen two to three month old male rats housed individually were divided into three groups of six animals based on the treatments (diets). The treatments consisted of control diet (no *Eupatorium*), 12.5% *Eupatorium*, and 25% *Eupatorium* in the diet. The diets were given for 3 weeks *ad libitum* and water was freely available. During the treatment period animals were observed for clinical symptoms and sick animal were killed. At the end of the feeding period all remaining animals were killed and samples of livers and all other major organs were fixed in 10% neutral formal-saline, embedded in paraffin wax and sectioned at 6 μm. Sections were stained by haematoxylin and eosin. The slides were examined by light microscopy.

RESULTS AND DISCUSSION

One week after beginning the study, a rat fed 25% *Eupatorium* showed anorexia, weakness and weight loss. This rat was killed and post-mortem examination carried out. The liver was pale with a nodular surface, and on histological examination had some proliferation of the bile ducts (Figure 1). In addition, enlargement of hepatocytes (megalocytosis) was seen (Figure 2).

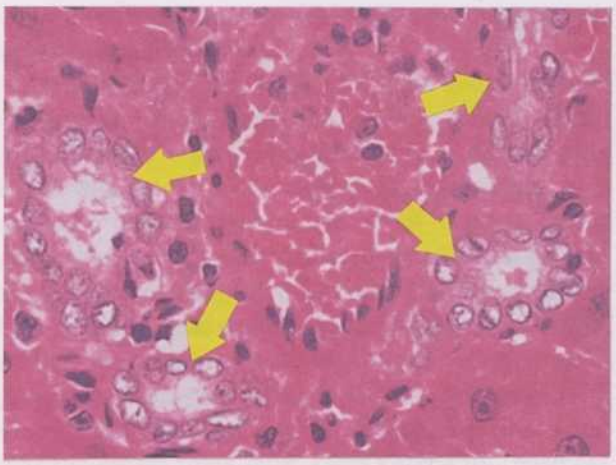

Figure 1. Bile duct proliferation in rat fed *E. inulifolium*. H & E Stain. 400 X

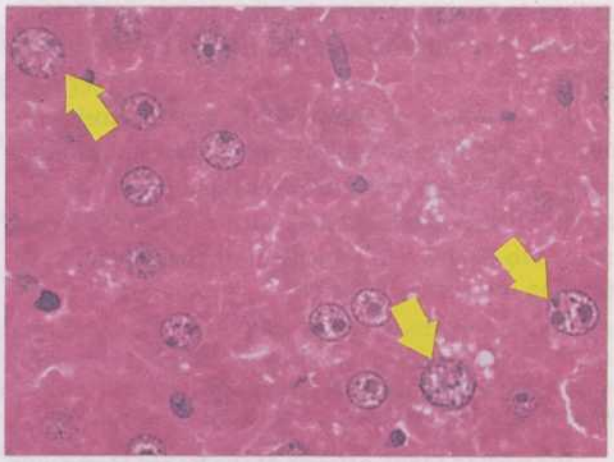

Figure 2. Megalocytes in liver of rat fed *E. inulifolium*. H & E Stain. 400 X

At the end of the three week feeding period all remaining rats were killed and gross and histopathological examinations carried out. No gross abnormalities were seen at necropsy, but at histopathological examination there was widespread megalocytosis of hepatocytes where both nuclei and cytoplasm were enlarged. Mild to moderate biliary proliferations were also observed. These microscopic changes were observed in all rats fed 12.5% and 25% *Eupatorium* but not in the control rats. No lesions were seen in other organs.

The microscopic pathology of the liver seen in the rats is consistent with but not diagnostic for toxicity caused by pyrrolizidine alkaloids (Hooper, 1978; Peterson and Culvenor, 1983).

Megalocytosis is seen in intoxication by other alkylating agents such as nitrosamines and aflatoxins. Nevertheless, most examples of this change are produced by the pyrrolizidine alkaloids (Jubb *et al.*, 1985). Megalocytosis is the result of an interaction between the antimitotic effect and the stimulus for regeneration of hepatocytes (Peterson and Culvenor, 1983; Cheeke and Shull, 1985). *Eupatorium* sp. also contains sequiterpene lactones, flavonoids and essential oils (Woerdenbag, 1986).

The range of histologic changes in rats fed *Eupatorium inulifolium* is different from that caused by other species of *Eupatorium*. *Eupatorium adenophorum* and *E. riparium* cause lung lesions in horses (O'Sullivan *et al.*, 1985) and *E. rugosum* causes a degeneration of the heart and skeletal muscles as well

as liver lesions (centrilobular degeneration with mild necrosis and fatty change) in horses (White *et al.*, 1985).

The histological findings of megalocytosis and bile duct proliferation in the liver of rats fed *Eupatorium inulifolium* indicated that this plant is hepatotoxic. Further feeding trials with *E. inulifolium* are needed to establish its toxicity in ruminants and to determine the tissues affected.

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