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TOXICITY OF VOLATILE COMPOUNDS OF *Senecio grisebachii* BAKER (margarita) FLOWERS, IN MICE

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SUMMARY

Intoxication caused by *Senecio spp* is characterized by an irreversible damage of the liver cells due to the action of pyrrolizidine alkaloids present in these plants. They contain other groups of volatile components, such as terpenes, which may also be toxic.

An aqueous extract obtained by water steam distillation of *Senecio grisebachii* fresh flowers was orally given to mice (0.5 mL/mouse/day) for 30 days. Animals were observed periodically. Weight loss, pale mucous membrane color, low body temperature and decreased capillary refill time were symptoms compatible with dehydration.

Histopathological findings showed congestion and edema in small and large intestines. Liver showed hydropic and fatty degeneration with centrilobular necrosis. Results indicate that terpenes alter membrane permeability regulation processes on the epitheliums of the digestive system

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and membranes of the hepatocytes, and that they might facilitate the intake of other substances, such as the hepatotoxic pyrrolizidine alkaloids.

Key words: *Senecio grisebachii, terpenes, intestine, liver.*

RESUMEN

TOXICIDAD DE COMPUESTOS VOLATILES DE FLORES DE *Senecio grisebachii* BAKER (margarita), EN RATONES.

La intoxicación causada por el género *Senecio* produce daño hepático irreversible, debiéndose esto a la acción de alcaloides pirrolizidínicos. Existen otros grupos de sustancias volátiles en la planta, como los terpenos, que también pueden ser tóxicos.

El extracto acuoso obtenido por destilación por arrastre con vapor de agua de flores frescas de *Senecio grisebachii* fue administrado por vía oral a ratones (0.5 mL/ratón/día) durante 30 días. Los animales fueron observados periódicamente. Pérdida de peso corporal, palidez de mucosas, temperatura corporal baja y tiempo de llenado capilar retardado fueron los síntomas compatibles con deshidratación.

Las observaciones histopatológicas evidenciaron congestión y edema a nivel de intestinos delgado y grueso. Enhígado las lesiones consistieron en degeneración hidrópica y grasa, con necrosis centrolobulillar. Los resultados indican la capacidad de los terpenos para alterar los mecanismos de regulación de permeabilidad de los epitelios del aparato digestivo y de las membranas celulares de los hepatocitos, lo que puede favorecer el ingreso de otras sustancias, como los alcaloides pirrolizidínicos hepatotóxicos.

Palabras clave: *Senecio grisebachii, terpenos, intestino, hígado.*

INTRODUCTION

Many plants can produce intoxication to humans or animals if eaten. Among them, *Senecio spp* is widespread around the world and it is known for its toxicological properties. In Argentina, 300 species inhabit the country (Gallo, 1987).

Different cases of intoxication in animals due to the ingestion of *Senecio spp* have been reported. In 1902, in Africa, a liver disease was related to *Senecio* consumption in cattle (Cushny and Watt, 1920). Other cases were recorded in England (Wilmot and Robertson, 1920), USA (Davis, 1957), Argentina (Carrillo *et al*., 1976; Venzano and Vottero, 1982; Araya *et al*., 1986), Uruguay (Podesta *et al*., 1977), Chile (Araya and González, 1979), Australia (Walker and Kirkland, 1981) and Brazil (Barros *et al*., 1987).

Consumption of tea prepared with leaves of *Senecio formosus* caused venous occlusion of the human liver in Colombia, where this plant is used for folk medicine (Gonzalez *et al*., 1997). Boiled rhizomes from *Senecio bonariensis* are used by some native tribes in the north of Argentina to treat skin diseases (Martinez Crovetto, 1964).

The toxicity of this plant is due to the presence of pyrrolizidine alkaloids (PAS), which were first identified in 1885 from *Senecio vulgaris* (Gradsal and Lasoux, cited by Morales, 1952). PAS are metabolized in the liver by means of hydrolysis, N-oxidation and demetilation. Metabolites derived from N-oxidation process mainly affect the liver leading to intense cellular alterations known as megalocitosis (Blood and Radostis, 1992). They also affect lungs and kidneys (Jubb *et al*., 1985), small intestine and central nervous system (Lloyd, 1957).

Other components present in *Senecio spp* are volatile substances like indoles and terpenes, which are part of the essential oil found in plants. It has been demonstrated that this kind of components can alter membrane permeability. Complex esters of sesquiterpenes alcohols and aromatic acids from *Ferula spp* caused an increment in membrane permeability to divalent versus monovalent cations (Abramov *et al*., 2001). The final consequence is the disruption of the ionic equilibrium between intra and extra cellular compartments.

Beta-myrcene (MYR), a monoterpene, causes skeletal alterations in mice fetuses from females fed on this substance during pregnancy (Delgado *et al*., 1993). Hepatic and renal injuries are induced by MYR in this species as well (Paumgarten *et al*., 1990).

In the north-east area, seven *Senecio spp* are common, being *S. grisebachii* (margarita) the most easily found. This plant is well adapted to different types of soils, especially sandy ones, which are, in fact, common in Corrientes, an Argentinean province. *Senecio grisebachii* is a 1 to 1.5 m shrub with dark green leaves and serrated edges. Flowers are daisy-like and bloom from October to November. This weed is common in areas where no farm activities have been lately performed and it is also easily found alongside roads. Its toxicity to animals has been reported in Argentina (Odriozola *et al*., 1997).

The aim of this research was to study the toxicological effects of the volatile components from *S. grisebachii* on liver and different areas of the mice digestive system.

MATERIAL AND METHODS

Plant material

Senecio grisebachii plants were gathered in summertime in Corrientes, located in the north-east region of Argentina. After botanic classification, plants tissues were separated into categories such as flowers, stems and leaves. Each fraction was stored for a few days in sterilized glass containers at refrigeration temperature (4 °C). Only flowers were used in this experience.

Extraction of volatile components

Four kilograms of flowers were boiled for approximately six hours in a glass flask connected to a refrigeration system in order to condense the steam to obtain the steam distillate. When heated up, the plant cells release their components and some of them are volatilized and carried by the steam. This method is frequently used for the extraction of the essential oil of plants. The aqueous distillate was collected in a sterilized glass container before being administered to mice.

Components identification

An aliquot of the extract was in turn extracted with n-hexane, to identify the distilled compounds. This was performed by GC-MS using a Hewlett-Packard 5890 Series II with a MSB model 5972 mass spectrometer provided by the 'Laboratorio de Química Orgánica', Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina. Beta-myrcene and other terpenes were identified. These data were presented in a previous study (Teibler *et al*., 2001).

Toxicological analysis

Three groups of five (5) CF1 mice were placed in cages. The distillate was mixed with drinking water (0.5 mL/mouse/day) for its oral administration to animals of the first two groups, for a period of 30 days. The third group only received water ad-libitum (controls). All the groups were fed with a commercial pellet diet ad-libitum.

Animals were observed periodically to determine the presence of any symptoms compatible with intoxication. Parameters measured were body temperature, weight, hydration (skin fold) and capillary refill time. After this time, animals were anesthetized and then euthanized to obtain tissue samples from liver and small and large intestines. They were fixed in 10% formalin, embedded in paraffin and processed according to the routine histological procedure, and 5 mm sections were stained with hematoxylin and eosin.

RESULTS

During the administration period, mice presented severe dehydration. Hair from perineal area was dirty, indicating that diarrhea contributed to the hydro-electrolytic alteration while in other areas hair was hirsute. Peripheral perfusion was altered, evidenced by pale mucous membrane color and a prolonged capillary refill time. Body temperature was decreased. After

necropsy, congestion was evident in the intestinal lumen. No other macroscopic alterations were perceived.

Microscopic lesions compatible with terpenes intoxication were found in the small and large intestines and in the liver. The apical portion of small intestine villi was congestive. Congestion was also evident in the large intestine, with dilation of lymphatic capillaries and edema in submucosa (Figure 1).

In the liver, congestion was observed in the sinusoids, with centrilobular hydropic and fatty degeneration as well as necrosis of the hepatocytes (Figure 2).

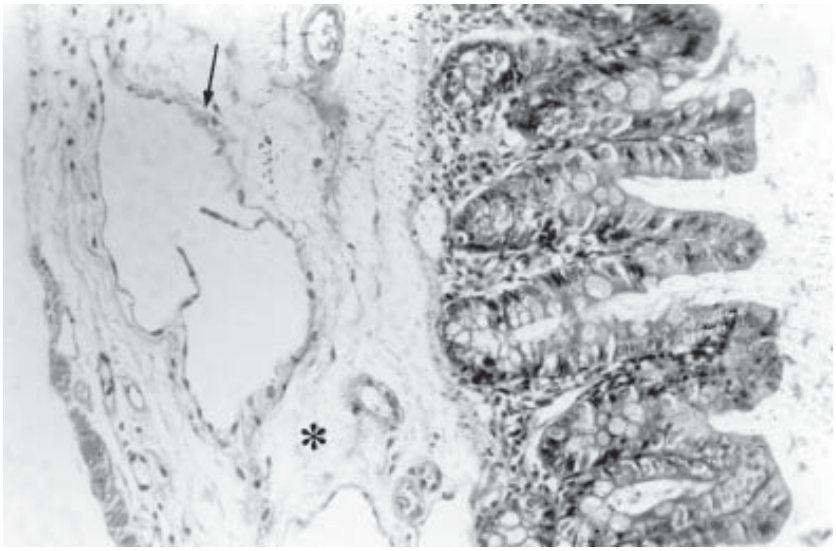

Figure 1. Histological section of the large intestine of a mouse treated with an aqueous distillate of *Senecio grisebachii* (0.5 mL po/day/mouse). Exposure time: 30 days. Edema of the submucosa (asterisk) and dilation of a lymphatic vessel (arrow) can be observed. Hematoxylin and eosin. 200X.

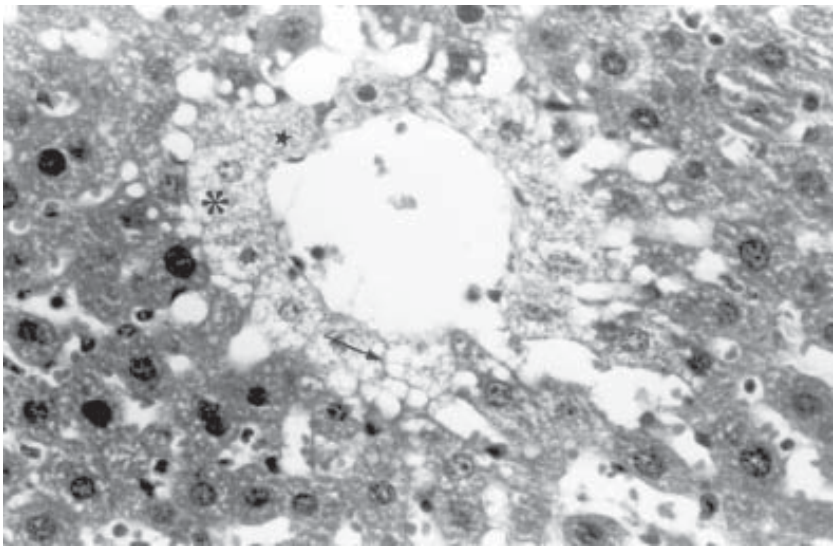

Figure 2. Histological section of the liver of a mouse treated with an aqueous distillate of *Senecio grisebachii* (0.5 mL po/day/mouse). Exposure time: 30 days. Hydropic (asterisk) and fatty (arrow) degeneration around the centrilobular area are observable, as well as necrosis of the hepatocytes (star). Hematoxylin and eosin. 400X.

DISCUSSION

The intestine epithelium is regarded as a selective barrier for the absorption of different substances. The results of this study indicate that the aqueous extract had a local irritant action over this barrier. This alteration on the membrane permeability allowed the absorption of components that are not absorbed under normal circumstances.

Terpenes are volatile plant components. Some of them are used as a defense mechanism since they perform a repellent activity against insects (Pare and Tumlinson, 1997). Furthermore, their odor and taste prevent them from being eaten by herbivores. Some

terpenes are also low-toxicity components. Cases of intoxication were reported in humans after ingestion of pine oil cleaners, which have terpenes in their composition. Symptoms are principally gastrointestinal due to gastric irritation after oral ingestion. Sometimes chemical pneumonitis is possible because terpenes are extensively absorbed in intestine and after distribution they remain in lung tissue (Brook *et al*., 1989).

Sesquiterpenes from *Ferula spp* may alter permeability to cations at the lipidic bilayer of cell and mitochondrial membranes. A terpene-mediated ion transport mechanism was described (Abramov *et al.*, 2001). The presence of the monoterpene beta-myrcene (MYR) has been previously evidenced as a volatile component from *Senecio grisebachii* (Teibler *et al*., 2001). This substance can increase the liver microsomal enzymatic activity, and it is considered as an inductor of the Cit-P450, which participates in the metabolism of many xenobiotics (De Olivera *et al*., 1997). MYR is also depicted as an embryo-toxic terpene for rats: 1.2 g/kg of this substance leads to growing retardation and skeletal anomalies in fetal skeletons (Delgado *et al*., 1993). Other researchers state that beta-myrcene has an analgesic effect as it is capable of inducing antinociception in mice, probably mediated by alpha-2-adrenoceptor release of endogenous opioids (Rao *et al*, 1990).

As far as the digestive system is concerned, beta-myrcene is toxic for the stomach and liver after its oral administration to mice. It is also highly irritant to the peritoneum, and deaths after intraperitoneal injection in rats and mice are possibly due to drug-induced chemical peritonitis (Paumgartten *et al*., 1990).

It is likely that MYR and other terpenes present in *Senecio grisebachii* alter membrane transport mechanisms as described above. This may justify the lesions observed in the intestines and liver presented in this study.

It is well known that *Senecio spp* contain pyrrolizidine alkaloids, which are capable of producing liver disease. They have an anti-mitotic activity and the continuous synthesis of DNA leads to an hepatic megalocytosis. Hepatic megalocytes are functionally

deficient and may die in an attempt to achieve mitosis (Lombardo de Barros *et al*., 1992).

Results indicate that terpenes alter membrane permeability regulation processes on the epitheliums of the digestive system and the hepatocytes, and that they might facilitate the intake of other substances, such as the hepatotoxic pyrrolizidine alkaloids; this should be confirmed with the administration of purified terpenes and pyrrolizidine alkaloids to mice, separated and together. These essays constitute the next step of this research.

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