



## Antidiarrhoeal effects of *Mikania glomerata* Spreng. (Asteraceae) leaf extract in mice

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**ABSTRACT:** *Mikania glomerata* Spreng. (Asteraceae) is a plant widely used to treat gastrointestinal disorders in Brazilian traditional medicine. In the present work, an aqueous extract of the leaves of *Mikania glomerata* has shown a decrease in the propulsive movements of the intestinal contents in mice. Oral administration produced an inhibition of gastrointestinal transit as effective as that produced by loperamide. These findings suggested that the aqueous extract of the leaves of *Mikania glomerata* might elicit an antidiarrhoeal effect by inhibiting intestinal motility.

**Keywords:** *Mikania glomerata*, Asteraceae, guaco, intestinal motility.

### INTRODUCTION

The World Health Organization (WHO) had established a special program for the Control of Diarrhoeal Diseases in the last two decades (WHO, 1994).

The majority of the world's population in developing countries still relies on herbal medicines to meet their health needs. Brazil has a great environmental and biological diversity compared with the rest of the world.

*Mikania glomerata* Spreng. (Asteraceae), popularly known as "guaco", is an herbaceous creeper and climbs over shrubs and trees. *M. glomerata*, commonly found in Brazil, is used in Brazilian folk medicine due to its respiratory tract effects (Penna, 1930). Moreover it is also used to treat cold, flu, fever, and rheumatism (Neves; Sá, 1991; Sá et al., 2003). It has been used in commercial preparations. The plant has local reputation as analgesic (Ahmed et al., 2001), antimutagenic (Arias et al., 1995), insecticidal (Arias et al., 1995), trypanomicidal (Arias et al., 1995, Muellas-Serrano et al., 2000), nutritive (Baidya et al., 1995); anticarcinogenic (Bishayee; Chatterjee, 1994), antiallergic (Fierro et al., 1999), antimicrobial (Hufford et al., 1998), and antiulcer (Paul et al., 2000) to *Mikania* sp. So far, some phytochemical studies had been reported on the kaurenoic and cinamoilgrandifloric acids, di and sesquiterpenes (Vilegas et al., 1997; Rüngeler et al., 2001), coumarins (Vilegas et al., 1997; Veneziani; Oliveira, 1999; Cabral et al., 2001), flavonoids and stigmaterol (Aguinaldo et al., 2003). Many phytochemical studies on *Mikania* species are described in the literature (Aguinaldo et al., 2003; Ahmed et al., 2001; Bardón et al., 1996; Bohlmann et al., 1981; 1982a e 1982b; Castro et al., 1989; Cruz; Roque, 1992; Cuenca et al., 1988, 1992, 1993; Diaz et al, 1992; Fabbri et al., 1997; Gutierrez et al., 1985, 1987, 1988; Herz et al., 1975; Herz; Kulanthaivel, 1985; Kiang et al., 1968; Knudsen et al., 1986; Lobitz et al., 1997, 1998; Nicollier; Thompson, 1981; Nunez et al.,

2004; Ohkoshi et al., 2004; Reis et al., 2003; Rungeler et al., 2001; Silva et al., 1984; Veneziani; Oliveira, 1999; Zamorano et al., 1995).

The present work was carried out to evaluate the anti-diarrhoeal potency of the aqueous leaf extract of *M. glomerata* Spreng. (Asteraceae) using intestinal motility test as experimental model in mice.

### MATERIAL AND METHODS

#### Plant material

Fresh leaves of *Mikania glomerata* were collected in the Herbarium of Medicinal Plants of the Faculdade de Ciências Farmacêuticas da UNESP in Araraquara, São Paulo, Brazil and have been kept in our laboratory for future reference. Botanical identity was kindly authenticated by Dr. LVS Sacramento of the Department of Active Natural Products - FCF - UNESP - Araraquara and a specimen of the plant has been deposited in the University Herbarium.

#### Preparation of aqueous extract

The plant material was air dried and then ground with 5 mm diameter mesh. The air-dried plant material was powdered through a 2 mm screen in a Wiley mill. The ground plant material was sequentially extracted by exhaustive maceration at room temperature with ethanol and water. The supernatants were filtered and evaporated under vacuum to obtain the aqueous extracts. The leaves aqueous extract was evaporated on a rotary evaporator and then reconstituted with sterile water (100 mg/mL).

#### Test for gastrointestinal motility (charcoal meal) in mice

Forty adult female Albino Swiss mice (*Mus*

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**Table 1.** Effect of orally administered 1000 mg/kg of *Mikania glomerata* aqueous extract on gastrointestinal motility in mice

Treatment	Distance of charcoal (%) $\pm$ S.D.
Control (veicule)	77.52 $\pm$ 8.97
Loperamide hydrochloride (5 mg/kg)	62.34 $\pm$ 11.21*
<i>Mikania glomerata</i> aqueous extract (1000 mg/kg)	66.99 $\pm$ 10.60*
<i>Mikania glomerata</i> aqueous extract (500 mg/kg)	77.41 $\pm$ 9.24

Values are mean  $\pm$  S.D., n = 10 (per group),

\* $P < 0.05$  vs. Control, Student's *t*-test

*domesticus domesticus*), weighing between 24-30 g were selected and housed in polypropylene cages (30 x 20 x 13 cm) in standard conditions (21  $\pm$  1°C with a 12 h reversed light-dark cycle and relative humidity 50-60%) for 10 days before performing the experiment. There was free access to water and normal commercial laboratory diet (Purina, Brazil). Experiments performed complied with the rules of the Institute of Laboratory Animal Resources, Commission on Life Sciences, and approved by the Ethical Committee of the Faculdade de Ciências Farmacêuticas - UNESP (Araraquara, São Paulo, Brazil - Protocol number 24/2004).

In the day of the test the animals were divided into four groups of ten mice each. They were weighed and deprived of food, with free access to water. Three hours after food deprivation the animals in treated groups (A and B) received orally *M. glomerata* extract (100 mg/mL) by gavage 500 and 1000 mg/kg, while controls groups received 0.9% NaCl sterile solution (Wong; Way, 1981; Olajide et al., 1999) and positive control group was given loperamide (5 mg/kg) as a reference antidiarrhoeal drug.

Ninety minutes after administering the extracts, 0.3 mL of a 5% charcoal suspension in 10% aqueous suspension of charcoal powder was administered to each animal orally. The animals were sacrificed 45 min later in CO<sub>2</sub> chamber and the abdomen opened. The percentage distances of the small intestine (from the pylorus to the caecum) traveled by the charcoal plug were determined. The method was described by Janssen; Jageneau (1957) and Wong; Way (1981).

### Statistical analysis

The results are expressed as means  $\pm$  S.D. Statistical significance was tested using a Student *t*-test. A difference was taken to be significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The standard method has used 18 - 24 h food

deprivation and then the animals were given an aqueous suspension of charcoal that causes animal stress (Janssen; Jageneau, 1957). These authors described the intestinal motility experiment using overnight fasted animals. In our protocol animals are deprived of food during 3h (Marona; Lucchesi, 2003). Our project is in agreement with the new concept and it is important for a respectful science.

Vermullen et al. (1997) observed rats with food deprivation. An experimental group of rats showed a decrease of -5% in body weight after 6-h fast. These animals showed -11% of liver weight. The 12-h deprived animals showed a decrease of -9.1% in body weight and -28% of liver weight. The third experimental group showed a -13% in body weight after 18-h fasting and -31.8% of liver weight. These observations could be a result of excessive stress caused by long fasting periods (Laties, 1987). Our work suggests only 3 hours of fasting before the experiment. This reduced time allows an experimental research carried out with non-stressed animals that could improve animal welfare and also increase the quality of science.

Our results suggest that *M. glomerata* aqueous extract showed lower percentages in small intestine distance in comparison with control group. In addition to providing promising new leads to the ongoing search for new drugs, the data analysis from this research has also suggested how future pharmacological investigation and ethnobotanical literature can be useful in guiding researches.

The distance of traveled by the charcoal plug is showed in Table 1. It is possible to compare that *M. glomerata* leaf extract (100 mg/kg) reduced significantly the distance of the charcoal marker in the feces compared with control group.

From this preliminary study this species was selected because of its intensive distribution in markets for gastrointestinal disorders and because it was available in good quantity in our laboratories.

Intestinal diseases are one of the main causes of death of infants particularly in developing countries

(WHO, 1994). It thus becomes important to identify and evaluate commonly available natural drugs as an alternative to currently used anti-diarrhoeal drugs.

In this protocol animals were deprived from food during a short time (3h) in agreement with Animal Welfare Guidelines as described by Marona and Lucchesi (2003). This short-time reduces aggression between mice and may be promoted as being beneficial to their physical and psychological health.

The results of this research reveal that the aqueous leaf extract of *Mikania glomerata* contains pharmacologically active substances(s) with antidiarrhoeal properties. This aspect may explain the use of *Mikania glomerata* as an antidiarrhoeal agent in popular medicine.

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