

Evaluation of the toxicity and antiulcerogenic activity of the ethanol extract of *Maytenus obtusifolia* Mart. leaves

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RESUMO: "Avaliação da toxicidade e atividade antiulcerogênica do extrato etanólico das folhas de *Maytenus obtusifolia* Mart." *Maytenus obtusifolia* é utilizada na medicina popular para o tratamento de úlceras graves, inflamações gerais e câncer. Apesar da importância etnofarmacológica desta espécie, nenhum estudo foi realizado para avaliar a sua toxicidade e atividade antiulcerogênica. Neste estudo, nós avaliamos a toxicidade e propriedade antiulcera do extrato etanólico das folhas de *Maytenus obtusifolia* (MO-EtOH). O MO-EtOH (10-1000 µg/mL) mostrou baixa toxicidade para as larvas de *A. salina* com CL_{50} maior que 1000 µg/mL. O MO-EtOH (2000 mg/kg, p.o.) não alterou o peso corporal e peso dos órgãos dos camundongos, mas foi observado um aumento no consumo de água dos machos e uma diminuição do consumo alimentar das fêmeas. Durante o estudo não foram observadas mortes e nem alterações macroscópicas nos órgãos dos camundongos. MO-EtOH (62,5, 125, 250 e 500 mg/kg) e lansoprazol (30 mg/kg) reduziram significativamente o índice ulcerativo para $65,58 \pm 8,74$, $43,00 \pm 9,53$, $15,50 \pm 7,56$, $54,75 \pm 8,88$ e $36,13 \pm 9,55$, respectivamente, em comparação com salina $82,13 \pm 12,48$. Em conclusão, o MO-EtOH apresentou baixa toxicidade e atividade antiulcerogênica, o que confirma o uso popular de *M. obtusifolia*.

Unitermos: *Maytenus obtusifolia*, Celastraceae, *Artemia salina*, toxicidade aguda oral, atividade antiulcerogênica.

ABSTRACT: *Maytenus obtusifolia* is used in folk medicine for the treatment of serious ulcers, general inflammations and cancer. Despite of the ethnopharmacological importance of this species, no study was conducted to evaluate its toxicity and antiulcerogenic activity. In this study, we evaluated the toxicity and antiulcerogenic property of the ethanol extract of the leaves of *Maytenus obtusifolia* (MO-EtOH). The MO-EtOH (10-1000 µg/mL) showed low toxicity for larvae of *A. salina* with LC_{50} higher than 1000 µg/mL. The MO-EtOH (2000 mg/kg, p.o.) did not change the body and organs weight of the mice, but it was observed an increase in the water consumption of males and a decrease in the food consumption of females. During the study no deaths and no macroscopic changes in the organs were observed in the mice. MO-EtOH (62.5, 125, 250 and 500 mg/kg) and lansoprazole (30 mg/kg) significantly reduced the ulcerative index for 65.58 ± 8.74 , 43.00 ± 9.53 , 15.50 ± 7.56 , 54.75 ± 8.88 and 36.13 ± 9.55 , respectively, in comparison with saline 82.13 ± 12.48 . In conclusion, the MO-EtOH showed low toxicity and antiulcerogenic activity, confirming the popular use of *M. obtusifolia*.

Keywords: *Maytenus obtusifolia*, Celastraceae, *Artemia salina*, acute oral toxicity, antiulcerogenic activity.

INTRODUCTION

The plants have acquired crucial importance in folk medicine, because of its therapeutic or toxic properties (Martins et al., 2003). The use of vegetal extracts in the treatment of some diseases is a widespread habit in Brazil. This habit may be explained, at least in part, by the belief that the plants exhibit therapeutic effect without causing toxic effects in the organism. Therefore, the search of medicinal plants has great

scientific importance, because it intends to validate the popular use and give greater safety (Brandão et al., 2006; Carvalho et al., 2008; Veiga-Junior et al., 2008).

In this context, a prominent family is Celastraceae, a large family of trees and shrubs. It is comprised by approximately 89 genera and 1300 species, with *Maytenus* as one of the largest genera, with some 270 species distributed throughout Brazilian territory (Stevens, 2001). Plants of this genus are used in South America's folk medicine like infusion or decoctions, due

to their analgesic, anti-inflammatory and antiulcerogenic activities (Côrrea, 1984; Bueno et al., 2005).

Several species of *Maytenus* have their biological activities proven experimentally. *Maytenus ilicifolia* one of the most studied species showed antinociceptive, anti-inflammatory (Jorge et al., 2004), antioxidant (Velloso et al., 2006; Melo et al., 2001) and antiulcer activities (Jorge et al., 2004). Antimicrobial (Kloucek et al., 2007) and antileishmanial activities (Perez-Victoria et al., 1999) were found in *M. macrocarpa*. It has also been demonstrated antimutagenic, antioxidant and antimicrobial properties of *M. krukovii* (Bruni et al., 2006) as well as inhibitory activity of the DNA polymerase β -lyase of *M. putterlickoides* (Feng et al., 2004). It was also documented an analgesic property for *M. aquifolium* (González et al., 2001) as well as antiulcerogenic activity of *M. robusta* (Andrade et al., 2007). *M. senegalensis* revealed antiplasmodial (Gessler et al., 1995; El Tahir et al., 1999), antibacterial (Matu & van Staden, 2003), antiviral activities (Otake et al., 1995) and antitumor property (Gessler et al., 1995).

In recent pharmacological studies our group showed that the ethanol extract of *Maytenus rigida* Mart. in the doses of 250, 500 and 750 mg/kg possesses anti-inflammatory, antiulcer and anti-diarrehoal activities (Santos et al., 2007).

M. obtusifolia is distributed in many states of the Northeast and Southeast of Brazil. It is popularly known as “carne-de-anta”, “carrancudo” or “bom-nome” and used in folk medicine like decoction from leaves for the treatment of serious ulcers (Corrêa, 1984), general inflammations and cancer (Agra et al., 2007). Grounded as powder from Stem-bark, it is used against external ulcers on the skin (Agra et al., 2007). The phytochemical screening showed the occurrence of three different chemical classes of secondary metabolites in *M. obtusifolia*, alkaloids for example *N*-methylflindersine, pentacyclic triterpenes as 3,4-secofriedelan-3-oic acid, 3b-hydroxy-11, 13-en-oleanane, 7-oxofriedelin, 3-oxo-29-hydroxyfriedelan, friedelin, 3b-hydroxy-9, 12-en-oleanane and 3b-hydroxy-9,12-en-ursane and flavonoids of the type 4'-O-methyl(-)-epigallocatechin, ouratea-proanthocyanidin A, catechin and epicatechin (Silva et al., 2008). Pharmacological studies demonstrated that this species possess analgesic (Freire, 1998) and neuroleptic activities (De Sousa & De Almeida, 2005).

Despite the popular use of this species, few pharmacological studies have been described in literature and there are no data on the toxicity of extracts of the leaves and their pharmacological effect on the gastric ulcer. Thus, the interest in this plant is justified by its potential medicinal value. Therefore, the aim of this study is to investigate the toxicity and antiulcerogenic activities of the ethanol extract obtained from the leaves of *M. obtusifolia*.

MATERIAL AND METHODS

Plant material and ethanol extract preparation

M. obtusifolia was collected in February, 2005, in Santa Rita, Paraíba, Brazil. It was identified by Dr. Maria de Fátima Agra, botanist from “Laboratório de Tecnologia Farmacêutica Prof. Delby Fernandes de Medeiros (LTF)/Universidade Federal da Paraíba (UFPB)” and a voucher specimen (Agra 3230) was deposited in the Herbarium Lauro Pires Xavier of the Department of Botany - UFPB, João Pessoa, Brazil. The leaves of *M. obtusifolia* were dried in oven at 45 °C for 3 to 4 days. After drying, the leaves (3,453.6 g) were powered and macerated with 95 % ethanol (EtOH) in three process of extraction, in intervals of 72 hours. The macerated leaves were filtered and concentrated under reduced pressure/rotaevaporator at 50 °C, resulting in the crude ethanol extract weighing 815.7 g (23.62 % on dry weight of the plant).

Animals

Male Swiss mice (28-35 g) were used to do *in vivo* experiments. They were obtained from the Biotery Thomas George of the LTF/UFPB. The animals were maintained in light/dark cycles of 12 h at 60 ± 1 % of humidity and temperature of 21.5 ± 2 °C, with food and water *ad libitum*. The experimental protocols were approved by the Institutional Committee of Ethics in Animal Research of the LTF/UFPB, registered under 0205/07.

Brine shrimp lethality test

To evaluate the toxicity of MO-EtOH, it was used the brine shrimp (*A. salina*) lethality test. 25 mg of eggs of *A. salina* were incubated in sea water (pH 8-9 and 29 °C) at artificial light during 24 h for occlusion of cysts and obtaining of the larvae. The extract was diluted in sea water (10-1000 μ g/mL) and then it was added 5 mL of different concentrations of MO-EtOH in tubes containing 13 to 15 nauplii. Three replications were done for each concentration and the experiment was repeated three times. The control group was prepared with the solvent and *A. salina*. The set was incubated at artificial light for 24 h and then the survivors larvae were counted to determine the LC_{50} (Lopes et al., 2002; Parra et al., 2001).

Evaluation of the acute oral toxicity

The acute oral toxicity study of *M. obtusifolia* was performed in mice. In this assay a single dose of 2000 mg/kg of MO-EtOH was orally administered (1 mL/100 g) to a group of twelve animals (six male and six female) after 6 h fast. Animals receiving the vehicle (saline solution 0.9 %) served as control. After treatment the behaviour parameters were observed, like

stimulatory, depressor effects and the death, during 30, 60, 90, 120, 180 and 240 minutes in the first day and once daily in the following 13 days to assess possible clinical or toxicological symptoms (Almeida et al., 1999). The body and organs weight, food and water consumption were evaluated in both sexes. At the end of the period of 14 days, the number of survivors was recorded for the determination of the LD₅₀. Macroscopic changes in the organs of the mice were evaluated.

HCl/ethanol-induced gastric lesions

The experimental was performed according to Mizui & Doteuchi (1981). After 24 h fasting, the mice (n = 6-8) received an oral administration of MO-EtOH at the respective doses of 62.5, 125, 250 and 500 mg/kg and positive control (lansoprazole, 30 mg/kg) or negative control (saline 0,9 %, 10 mL/kg). Fifty minutes after the treatment, all mice received 0.2 mL of 0.3 M HCl/ethanol 60 % to induce gastric ulcer. The animals were killed 1 h after treatment with the ulcerogenic agent and the stomachs were removed and opened along the grater curvature. The ulcerative index (UI) was calculated according to the methodology described by Szelenyi & Thiemer (1978).

Statistical analysis

The results are expressed in mean ± S.D. Differences between the means were statistically compared using Student's t-test for acute toxicity and one-way analysis of variance (ANOVA) followed by Dunnett's test for the antiulcerogenic activity. The values

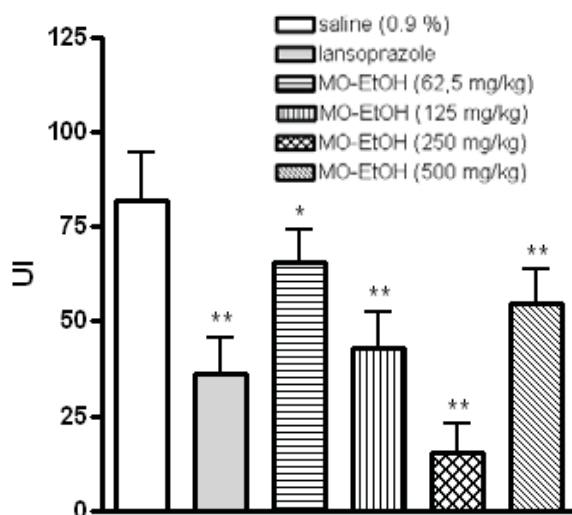


Figure 1. Effect of the ethanol extract obtained from *M. obtusifolia* on UI. The results are expressed as mean ± SD for six or eight mice. Statistical comparison was performed using ANOVA followed by the Dunnett's test. * $p < 0.05$ and ** $p < 0.01$ in comparison with saline 0.9 %.

were considered significantly different when the levels of $p < 0.05$. The software used was the GraphPad Prism[®] 4.03 software (GraphPad Software Inc., San Diego CA). The LC₅₀ for brine shrimp lethality test was determined according to the statistical method of Probitos using the program Microcal Origin 6.0.

RESULTS AND DISCUSSION

M. obtusifolia is used in folk medicine for the treatment of serious ulcers (Corrêa, 1984), general inflammations, cancer (Agra et al., 2007) and against external ulcers on the skin (Agra et al., 2007). However, very little is known about their toxicity. For this reason, research is carried out in order to determine the pharmacological action and toxicity of this plant (Parra et al., 2001).

In the present study, the toxicity of the MO-EtOH by the brine shrimp lethality test was examined. This bioassay was performed three times and the LC₅₀ of these three tests were higher than 1000 µg/mL (Data not shown). Thus, the MO-EtOH showed low toxicity for larvae of *A. salina*.

Additionally, we evaluated the acute oral toxicity of the ethanol extract and we observed that the single dose of 2000 mg/kg of the MO-EtOH induced analgesia in the second and third hours after oral administration in the mice after administration, this effect was reversible in the fourth hour after treatment. This result is consistent with those obtained for Freire (1998), in which *M. obtusifolia* showed analgesic activity. During the 14 days of observation no deaths were showed and at the end of the experiment no macroscopic changes in the organs were observed in the animals.

In addition to these parameters, the evolution body weight was evaluated, in which changes are an indicator of adverse side effects, as the animals that survive cannot lose more than 10 % of the initial body weight (Raza et al., 2002; Teo et al., 2002). The water and food consumption were also evaluated, which are important parameters in the study of safety of a product with therapeutic purpose (Iversen & Nicolaysen, 2003).

In the present work, MO-EtOH did not induce changes in the body and organs weight of the mice of both sexes, water consumption of the females and food consumption of the males when compared with their respective control group (Table 1). But an increase in water consumption of the male mice treated with the extract was observed (Table 1). This result may be related with an increase in the diuresis, which leads to much urinary volume and consequently more water consumption. A decrease in food consumption of the females was showed (Table 1) without, however, changing the weight of the mice when compared with the saline group. This finding may be due to a decrease in the metabolism of the females. These results suggest that the ethanol extract obtained from the leaves of the

M. obtusifolia shown low toxicity to mice treated orally in the tested dose, thus ensuring safety for the research of the antiulcerogenic activity, with intention to prove the traditional use of this plant.

Peptic ulcer is a common disorder of the entire gastrointestinal tract (Maity et al., 2003). It occurs mainly in the proximal duodenum and stomach. When located, this is called gastric ulcer, which are deep necrotic lesions involving the entire mucosal depth and the muscularis mucosae (Chow et al., 1998; Tarnawski, 2005). Gastric ulcer has been centralized on an imbalance between some endogenous and exogenous aggressive (hydrochloric acid, reactive oxygen species, ethanol, *Helicobacter pylori*, steroidal and nonsteroidal antiinflammatory drugs, among others) and defensive factors (mucus-bicarbonate barrier, surface active phospholipid, prostaglandin, mucosal blood flow, cell renewal and migration, antioxidants and antioxidative

enzymes and some growth factors) (Bandyopadhyay, 2001; Bhattacharjee et al., 2002; Liu & Crawford, 2005).

The antiulcerogenic activity was evaluated through oral administration of HCl/ethanol in mice, which causes necrotizing lesions in the gastric mucosa. These lesions are caused by decrease of mucous layer and the increase of acid secretion (Mizui & Doutechi, 1981). These factors occur because that agent causes oxidative stress and lipid peroxidation and DNA fragmentation, resulting in ulcerative lesions (Gonzales et al., 2001). This model evaluates the capacity of the drugs to protect the gastric mucosa, making this technique suitable for investigation of natural products antiulcer activity.

In the present study the HCl/ethanol solution induced both long ulcers and petechial lesions within a relatively short time, with UI of the saline 0.9 % group of 82.3 ± 12.48 . Pretreatment with MO-EtOH

Table 1. Effect of the ethanol extract obtained from *M. obtusifolia* (2000 mg/kg, p.o.) on body and organ weights and water and food consumption in mice.

	Control (saline 0.9%)	MO-EtOH (2000 mg/kg)
Body weight (g)		
Female		
Initial	34.08 \pm 2.91	32.22 \pm 4.14
Final	37.88 \pm 3.92	35.32 \pm 3.84
Increased (%)	09.48 \pm 5.21	08.91 \pm 2.96
Male		
Initial (g)	35.92 \pm 1.66	33.98 \pm 1.91
Final (g)	40.90 \pm 2.89	38.78 \pm 2.58
Increased (%)	11.99 \pm 4.22	12.25 \pm 4.28
Organ weight (g)		
Female		
Heart	0.16 \pm 0.02	0.16 \pm 0.04
Liver	1.81 \pm 0.32	1.74 \pm 0.36
Kidney	0.51 \pm 0.10	0.48 \pm 0.06
Male		
Heart	0.19 \pm 0.01	0.18 \pm 0.01
Liver	2.31 \pm 0.21	2.18 \pm 0.28
Kidney	0.53 \pm 0.04	0.50 \pm 0.05
Water consumption (g)		
Female	12.30 \pm 1.84	12.24 \pm 2.00
Male	09.74 \pm 0.86	10.62 \pm 1.04*
Food consumption (g)		
Female	08.33 \pm 0.91	07.09 \pm 1.26**
Male	06.61 \pm 0.96	06.52 \pm 0.82

Data are expressed as mean \pm S.D. for six mice. Statistical comparison between saline 0.9 % and MO-EtOH 2000 mg/kg was performed using Student t-test. * $p < 0.05$ and ** $p < 0.01$ in comparison with the saline 0.9 %.

(62.5, 125, 250 and 500 mg/kg) and lansoprazole (30 mg/kg), significantly reduced the UI for 65.58 ± 8.74 , 43.00 ± 9.53 , 15.50 ± 7.56 , 54.75 ± 8.88 and 36.13 ± 9.55 , respectively, when compared with the saline 0.9 % group. The MO-EtOH induced a significant protective effect in variable degrees (Figure 1). This result indicates that the plant in study has antiulcerogenic activity. It is consistent with those obtained by Santos et al., (2007), in which the ethanol extract of *M. rigida* (125, 250, 500 and 750 mg/kg) showed antiulcer activity against gastric lesions induced by ethanol in rats. It was also similar to the results observed in the studies undertaken with other species of *Maytenus*.

CONCLUSION

The results of this study suggest that the ethanol extract obtained from the leaves of *M. obtusifolia* show low toxicity to both *A. salina* and mice. The MO-EtOH displays gastroprotective activity, as demonstrated by its significant inhibition of the formation of ulcers induced using the HCl/ethanol model; however, more specific studies must be carried out to elucidate the mechanisms involved in this activity. The data obtained confirmed the popular use of this plant.

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