

ACUTE TOXICITY AND DECREASED PERISTALSIS IN MICE CAUSED BY *TAXODIUM MUCRONATUM* AND *ACACIA FARNESIANA* EXTRACTS

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(Received May 2012; Accepted September 2012)

ABSTRACT

Diarrheal diseases remain a health problem in Mexico and the world. *Taxodium mucronatum* Ten. (Taxodiaceae) (Ahuehuete, Sabino) and *Acacia farnesiana* (L.) Will. (Leguminosae) (Huizache) are popularly used in the treatment of diarrhea. The aim of this work was to determine, *in vivo*, the lethal media doses and effect on intestinal propulsion of aqueous and ethanol extracts from leaves and small stems in mice. The lethal media doses were estimated using the Lorke method, modified by the Reed-Müench approach, and probit units; the results indicated a weak acute toxicity level. The effects on intestinal propulsion were tested using the charcoal meal method and we found that the extracts inhibited the movement of the gastrointestinal content. The data explain in part the medicinal use of *T. mucronatum* and *A. farnesiana*, and support their use as antidiarrheal agents. www.relaquim.com

Key words: *Taxodium mucronatum*, Ahuehuete, *Acacia farnesiana*, Huizache, lethal media dose, antidiarrheal.

RESUMEN

Las enfermedades diarreicas siguen siendo un problema de salud en México y el mundo. *Taxodium mucronatum* Ten. (Taxodiaceae) (Ahuehuete, Sabino) y *Acacia farnesiana* (L.) Will. (Leguminosae) (Huizache), se usan popularmente en el tratamiento de la diarrea. El objetivo de este trabajo fue determinar, *in vivo*, la dosis letal media y el efecto sobre la propulsión intestinal en ratones, de los extractos acuoso y etanólico de hojas y tallos pequeños de *T. mucronatum* y de *A. farnesiana*.

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Las dosis letales medias se estimaron por el método de Lorke modificado tomando en consideración los planteamientos de Reed- Muench y el uso de unidades probi-tas, los resultados indicaron un débil nivel de toxicidad aguda de estas especies. El efecto sobre la propulsión intestinal se probó utilizando el método de carbón activado, se encontró que ambos extractos inhibieron el avance del contenido gas-trointestinal. Los resultados contribuyen a explicar el uso medicinal del Ahuehuete y del Huizache como agentes antidiarreicos. www.relaquim.com

Palabras clave: *Taxodium mucronatum*, Ahuehuete, *Acacia farnesiana*, Huizache, dosis letal media, antidiarreico.

INTRODUCTION

Diarrheal diseases continue to be a cause of morbidity and mortality worldwide (WHO-SIS, 2009; WHO, 2009; UNICEF, 2009). In Mexico, in 2005, infectious intestinal diseases (including diarrhea) were included in the top 20 causes of death in men, women, and people over 65 years of age (SS, 2005).

Taxodium mucronatum Ten. (Taxodiaceae) (Ahuehuete, Sabino) (synonymy with *Taxodium montezumae*, *Taxodium mexicanum*). Ahuehuete is a tree that is mentioned in the Florentine Codex. Its name is a nahuatl word that translates to “old man from the water”, it is a millenary species linked to Mexican history. The Ahuehuete is 20 to 30 m high, its trunk is thick and frequently divided from the base, it has a brown-reddish bark. The branches form a wide crown, with hanging twigs and linear sheets (Rzedowski and Rzedowski, 1991). In Mexican traditional medicine, it is used to treat diarrhea as well as skin diseases, sores, hemorrhoids, burns (Argueta, 1994). It contains biflavones (Ishratullah *et al.*, 1978), amentoflavone and its derivatives (Pan *et al.*, 2005), flavonoids, quercetin glucoside, and a diterpenoid (Argueta, 1994).

Acacia farnesiana (L.) Willd. (Leguminosae) (Huizache, Cascalote, Colita, Espino blanco), (synonymy with *Acacia aciculans*, *Mimosa farnesiana* and *Vachellia farnesiana*). Huizache is a tree or shrub, 2 to 5 m in height, its trunk is highly branched, stipules has shaped thorns, short-petioled

leaves, 2 to 6 cm long (Rzedowski and Rzedowski, 1990), the flowers are fragrant, yellow, and its fruits are cylindrical dark pods; this species is distributed throughout the country, mainly in arid and semiarid regions (Argueta, 1994). *A. farnesiana* is used in the Mexican traditional medicine for the treatment of diarrhea, dysentery, diabetes, wounds, cough, and typhoid (Argueta, 1994). *A. farnesiana* contains 7, 3' dihydroxy-4' methoxy flavone (farnisin) (Sahu *et al.*, 1998), cyanogens (linamarin and lotaustralin) (Seigler *et al.*, 1979), tannins (Wassel *et al.*, 1990), kaempferol and its derivatives (El-Negoumy *et al.*, 1982), naringenin 7-O- β -(4''6''-digalloylglucopyranoside), quercetin 7-O- β -(6''-galloylglucopyranoside), myricetin 7-O- β -(6''-galloylglucoside), kaempferol 7-(6'' galloylglucoside) (Barakat *et al.*, 1999), among other compounds.

Both species, *Taxodium mucronatum* and *Acacia farnesiana*, are used to treat diarrhea, in aqueous decoction from leaves and small stems.

There are drugs that have proved to be effective to treat diarrhea (loperamide, diphenoxylate); however, they have shown adverse effects (Burks, 1998; Guststein and Alkil, 2003; Jafri and Pasricha, 2003) and they are not readily available for the majority of the population due to their price. For these reasons, it is necessary to undertake the study of plants whose ethnobotanic references point them out as species with action against diarrhea. It is imperative

to explore their toxicity degree given that not all species are innocuous. Moreover, considering that the common factor in diarrheas is an increased intestinal motility, research on this issue must be done. The aim of the present work was to determine the acute degree of toxicity, as well as the effect on the gastrointestinal motility in mice of two medicinal plants currently used for the treatment of diarrhea in Mexico.

MATERIAL AND METHODS

Plant material. Leaves and small stems from *Taxodium mucronatum* Ten. were collected in Mexico City and leaves and small stems from *Acacia farnesiana* (L.) Willd. in Hidalgo State, Mexico. These specimens were taxonomically identified by Biol. Alfredo Patiño-Siciliano (*Departamento de Botánica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional*). Voucher specimens were deposited in the IEB Herbarium (*Centro Regional del Bajío, Instituto de Ecología, A. C., Pátzcuaro, Mich.*) [*T. mucronatum*: IEB 007069; *A. farnesiana*: IEB 071574].

Animals. Swiss albino mice (20-25 g, NIH) maintained under standard conditions and fed with standard diet and water *ad libitum* were used in all experiments. The animals were treated in compliance with the national and international recommendations (Olfert *et al.*, 1993; SS, 1999; Lomeli, 2002), routinely used in our laboratory.

Materials. Loperamide (Sigma-Aldrich, Inc., St. Louis, MO, USA), Arabic gum (Química Meyer), charcoal meal (Hycel Mexico). Other chemicals were analytical grade.

HPLC analysis

Sample: Quercetin, aqueous and ethanol extracts of leaves and twigs of *T. mucronatum* and *A. farnesiana*. Chromatographic conditions: Column Symmetry C18 5 μm (4.6 \times

250 mm). DAD detector at 270 nm. Oven temperature 30°C. Injection volume of 10 μL . Mobile phase: A: Phosphoric acid 3% (v/v). B: MeOH; C: ACN. Elution gradient: inicio 100% A; 10 min, 85% A, 15% C; 30 min, 70% A, 10% B, 20% C; 40 min, 10% A, 15% B, 75% C; 55 min, 5% A, 15% B, 80% C; 56 min, 100% A; 66 min 100% A. Flow: 1 mL/min).

Extract preparation

Dried and powdered leaves and small stems of each plant were Soxhlet extracted with distilled water. The extract was filtered through gravity and concentrated in a rotary evaporator Buchi RE-111, and finally exposed to an air current at room temperature (22 ± 2 °C), until a dark brown semisolid mass was formed (yield 16.8% dry weight of *T. mucronatum* and 25.4% dry weight of *A. farnesiana*). Ethanol extracts were prepared in ethanol (96%) maceration until a dark green semisolid mass was formed (yield 8.7% dry weight of *T. mucronatum* and 16.8% dry wt. of *A. farnesiana*).

Plant extracts were freshly prepared each time and administered at a volume of 0.1 mL/10 g body weight for all the pharmacological tests.

Acute toxicity test

The following was administered i.p. to groups of three mice: isotonic saline solution to the control group. The test group dosing was given according to that established by Lorke (1983). The 48-hour mortality was registered and the survivors were kept in observation for the next 15 days.

Taking into account the Lorke method (1983), modified by the Reed-Müench approach (Pouillot *et al.*, 2003), and the use of probit analysis. The equation to determine the lethal media doses (LD_{50}):

$$\log LD_{50} = \left[\frac{P_{50} - P_a}{P_b - P_a} \right] \log \left(\frac{b}{a} \right) + \log(a)$$

[*a*: dose < 50% mortality; *b*: dose > 50% mortality; P_{50} : probit units at 50% morta-

lity; P_a : probit units at dose a ; P_b : probit units at dose b].

Intestinal motility test

Mice were starved for 24 h prior to the experiment, but were allowed free access to water. Extract was (1-300 mg/kg) (Astudillo-Vázquez *et al.*, 2008) orally administered to animals ($n = 10$). Loperamide (5 mg/kg, *po*) was given to animals in the reference group while control animals received isotonic saline solution. Thirty minutes after drug administration, a 10% charcoal suspension in a 5% aqueous suspension of arabic gum was *po* administered to each animal. After thirty minutes, the animals were killed by cervical dislocation and the entire length of the intestine (from the pylorus to the cecum) was removed carefully. The distance travelled by the charcoal plug in the intestine (A) and the total length of the intestine (B) was measured (Williamson *et al.*, 1996). The percentage of intestinal transit was calculated for each mouse as $(A/B) \times 100$.

Statistical analysis

The results were statistically evaluated using a one-way ANOVA followed by Dunnett's t test ($p < 0.05$).

RESULTS AND DISCUSSION

The results obtained for the LD_{50} from the extracts of *T. mucronatum* and *A. farnesiana* (Table 1) show that they are weakly toxic according to classifications based on this parameter (Déciga-Campos *et al.*,

2007). This means that they do not induce immediate toxicity in the tested experimental conditions. Also the pharmacologically active doses were lower than lethal media doses obtained. It has been reported that the genus *Acacia* is included in the wide group of plants that contain cyanogens (Seigler *et al.*, 1979). LD_{50} results suggest that *A. farnesiana* does not have or has low concentrations of cyanogen compounds.

The results show that the extracts of both the Ahuehuete and the Huizache were able to diminish the intestinal motility; with all the extracts a rising tendency of the inhibitory effect on the gastrointestinal motility was observed when the doses were increased (Tables 2 and 3). Inhibition percentages of peristaltic motion at the highest dose tested (300 mg/kg) for both extracts were 30% higher as compared to the control group. This level is considered appropriate for the total extract, considering that the population consumes medicinal teas of both species to treat diarrhea. In this study, the charcoal meal method was selected to follow the displacement of the gastrointestinal content because reduction of gastrointestinal motility is one mechanism by which many antidiarrheal agents can act (Akah *et al.*, 1999; Astudillo-Vázquez *et al.*, 2009); this may be the case of both *Taxodium mucronatum* and *Acacia farnesiana*. Phytochemical studies of the Ahuehuete (Ishratullah *et al.*, 1978, Argueta, 1994; Pan *et al.*, 2005) and the Huizache (Barakat *et al.*, 1999), have detected that they contain flavonoids among other compounds.

One of the flavonoids present in *A. farnesiana* is kaempferol (El-Negoumy *et al.*, 1982), which is a compound with antispasmodic activity in guinea pig isolated ileum (Trute *et al.*, 1997) and its mechanism is exerted through calcium mobilization (Capasso *et al.*, 1991). Furthermore, the mucilage that contains *A. farnesiana* gives body and volume to feces by favoring water absorption, which may contribute to decrease the amount of liquid feces.

Table 1. Lethal media dose of total extracts from *Taxodium mucronatum* and *Acacia farnesiana*, tested in mice.

Plant	LD_{50} (g/kg)	
	Aqueous Extract	Ethanol Extract
<i>T. mucronatum</i>	3.289	2.154
<i>A. farnesiana</i>	1.499	0.775

$n = 10$

Table 2. Effect of aqueous extracts from *T. mucronatum* and *A. farnesiana* on gastrointestinal transit of a charcoal meal in mice.

Treatment	Dose (mg/kg, po)	n	Charcoal meal advance (%)	Inhibition (%)
Control (isotonic saline solution)	-----	10	82.7579 ± 3.0236	-----
<i>T. mucronatum</i> extract	3	10	80.4700 ± 3.7837	2.76
<i>A. farnesiana</i> extract	3	10	69.3080 ± 2.6831*	16.25
<i>T. mucronatum</i> extract	30	10	61.5700 ± 2.2641*	25.60
<i>A. farnesiana</i> extract	30	10	60.1600 ± 2.9611*	27.31
<i>T. mucronatum</i> extract	300	10	56.0635 ± 2.6790*	32.26
<i>A. farnesiana</i> extract	300	10	56.5182 ± 3.0511*	31.71
Loperamide	5	10	56.2057 ± 2.0023*	32.08

*p < 0.05 respect to the control group.

Table 3. Effect of ethanol extracts from *T. mucronatum* and *A. farnesiana* on gastrointestinal transit of a charcoal meal in mice.

Treatment	Dose (mg/kg, po)	n	Charcoal meal advance (%)	Inhibition (%)
Control (isotonic saline solution)	-----	10	83.5364 ± 3.0274	-----
<i>T. mucronatum</i> extract	3	10	77.5750 ± 3.4116	7.14
<i>A. farnesiana</i> extract	3	10	61.1933 ± 3.7715*	26.74
<i>T. mucronatum</i> extract	30	10	68.8875 ± 2.4481*	17.53
<i>A. farnesiana</i> extract	30	10	57.4300 ± 2.9373*	31.25
<i>T. mucronatum</i> extract	300	10	47.5100 ± 3.1577*	43.13
<i>A. farnesiana</i> extract	300	10	54.6600 ± 3.6590*	34.57
Loperamide	5	10	56.2264 ± 2.0042*	32.69

*p < 0.05 respect to the control group.

On the other hand, quercetin derivatives are present in *A. farnesiana* (Barakat *et al.*, 1999) and *T. mucronatum* (Argueta, 1994), this flavonoid inhibits the contractile responses in guinea pig isolated ileum (Lutterodt, 1989), probably by reducing the available calcium concentration (Capasso *et al.*, 1991). More research is needed to elucidate the mechanism by which the extracts work. Of course, it is also necessary to isolate the active compounds. HPLC analysis of the extracts studied did not indicate the presence of quercetin, but it showed other aromatic compounds, perhaps biflavonoids, however this cannot be stated conclusively.

CONCLUSIONS

Our results suggest that the extracts ob-

tained from the species studied do not induce immediate toxicity in the tested experimental conditions.

The obtained data present evidence that supports the use of *T. mucronatum* and *A. farnesiana* as antidiarrheal agents in Mexican traditional medicine.

ACKNOWLEDGEMENTS

The authors thank Biol. Alfredo Patiño Siciliano for the taxonomic identification of the plant material. Thanks to Prof. Daniel Villegas Estrada for mathematical assistance. A. Astudillo-Vázquez is thankful for the COFAA-IPN grant. Sponsors: SIP-IPN (20121460) through partial support.

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