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Teratogenic Effects of *Mimosa tenuiflora* in a Rat Model and Possible Role of *N*-Methyl- and *N,N*-Dimethyltryptamine

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ABSTRACT: *Mimosa tenuiflora* is a shrub/tree found in northeastern Brazil sometimes eaten by livestock and believed to be responsible for malformations observed in many animals from that region. The teratogenic compounds in *M. tenuiflora* are not known. This study used pregnant rats fed *M. tenuiflora* and components therefrom for bioassay and fractionation of possible teratogenic compounds. Rat pups were examined for cranial−facial defects and skeletal malformations. Experimental diets included *M. tenuiflora* leaf and seed material, extracts of leaf and seed, alkaloid extracts of leaf and seed, and *N*-methyltryptamine and *N,N*-dimethyltryptamine. Pups from mothers who received *M. tenuiflora* plant material, methanol extracts, alkaloid extracts, and purified *N*-methyltryptamines had a higher incidence of soft tissue cleft palate and skeletal malformations. Results are summarized as to the frequency of observed cleft palate and other noted malformations for each diet versus control.

KEYWORDS: *N-methyltryptamine, N,N-dimethyltryptamine, Mimosa tenuiflora, teratogen, cleft palate*

INTRODUCTION

Mimosa tenuiflora is a common shrub/tree found in many parts of South America and northward into Mexico. The plant has been used for a variety of psychoactive purposes by humans.[1] In northeastern Brazil it is often eaten by livestock including goats, sheep, and cattle and is believed to be responsible for reported fetal malformations observed in many animals from that region. In a goat model the fetal malformations have been characterized by cleft lip, cleft palate, unilateral corneal opacity, ocular bilateral dermoids, buphthalmos, and segmental stenosis of the colon.[2] Even though such cases of teratogenicity have been associated with the plant, *M. tenuiflora* is an accepted and utilized forage plant in many regions. The teratogenic compound(s) in *M. tenuiflora* are not known, and thus a rat model was developed to test for possible putative toxins.[3] In the previous work, mimosa seeds were fed to an experimental group of pregnant rats during gestation days 6−21 as a formulated feed containing 10% *M. tenuiflora* seed. In comparison to the control group, which was fed the same ration without seeds, there were no differences observed in weight gain or food and water consumption; however, there were statistically significant differences in the number of skeletal malformations in pups from those dams receiving a ration containing 10% *M. tenuiflora* seeds. The malformations reported included cleft palate, scoliosis, missing or extra bones, or misaligned bones of the sternebrae, the caudal, lumbar, sacral, and thoracic vertebrae, metacarpals, ribs, and parietal bones. On the basis of the observed effects from this initial investigation it was concluded that the rat model would be useful to study the teratogenicity of *M. tenuiflora* and its active principle. The objective of this study was to further explore the active teratogenic principles of *M. tenuiflora* in the rat model. Specifically this study examined the effects of feeding mimosa leaf, seed, crude methanolic extracts of leaf and seed, the crude base (alkaloid) extracts of leaf and seed, and two major tryptamine alkaloids found in *M. tenuiflora* on induction of cranio−facial abnormalities and malformations of the skeletal system in rat pups.

MATERIALS AND METHODS

General Experimental Procedures. NMR spectra were recorded on a JEOL 300 spectrometer in $CDCl_3$ with the chemical shift referenced to the residual $CHCl_3$ resonance at 7.26 ppm. HPLC-UV-MS experiments were performed using a Thermo Finnigan LCQ Advantage Max mass spectrometer, a Surveyor Plus autosampler, a Surveyor MS pump plus, and APCI ionization source (Thermo Finnigan, San Jose, CA, USA). APCI operating conditions were optimized for maximum ion signal using a standard solution of reserpine and the protonated molecular ion at m/z 609. Flash chromatography was performed using a Biotage Isolera I system (Biotage, Charlotte, NC, USA).

Chemicals and Reagents. All commercially purchased solvents or reagents were of analytical reagent grade unless otherwise noted. Tryptamine (98%), *N*-methyltryptamine (99%), and sodium triacetoxyborohydride (95%) were purchased from Aldrich Chemical (Milwaukee, WI, USA). Acetonitrile was of HPLC grade, and water was Millipore purified (18.2 MΩ·cm).

Biological Materials. *M. tenuiflora* leaf and seed materials were collected in northeastern Brazil, near Patos, Paraiba, Brazil, and identified by one of the authors (F.R.-C.). No voucher specimen was recorded as this tree is common throughout northeastern Brazil, and it is easily identified.

Animal Model, Feed Formulation, and Testing. The experimental model was previously described in detail as to animal use, feed ration preparation, and general method of analysis.[3] In general, pregnant rats were fed a formulated rat chow ration containing

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Table 1. Number of Dams, Number of Pups/Dam, and the Proportion (Mean Percent ± SEM) of Rat Pups with Various Malformations When Pregnant Dams Were Dosed with Various *Mimosa tenuiflora* Plant Parts, Extracts, or Purified Compounds

treatment	no. of dams	mean no. of pups/dam	NMT in feed ration (μg/g)	DMT in feed ration (μg/g)	cleft palate (soft tissue) (%)	hard palate damage[a] (%)	skeletal deformities[b] (%)	scoliosis[c] (%)
control	7	12.8			0 ± 0	0 ± 0	9.0 ± 5.9	1.2 ± 1.0
leaf	9	12.1	149	154	27.5 ± 5.0	0 ± 0	25.3 ± 5.9	1.2 ± 1.1
leaf (MeOH)[d]	7	13.1	134	139	24.9 ± 4.8	0 ± 0	38.4 ± 6.6	3.47 ± 2.5
leaf alkaloid	6	10.8	120	108	25.1 ± 3.6	0 ± 0	53.2 ± 8.9	0 ± 0
seed	11	13.7	11	8	26.5 ± 5.9	0 ± 0	37.9 ± 6.2	4.0 ± 1.7
seed MeOH	7	14.0	8	6	3.1 ± 2.2	0 ± 0	50.3 ± 7.2	1.3 ± 1.3
seed alkaloid	7	11.6	9	4	10.1 ± 4.6	0 ± 0	40.2 ± 4.4	2.8 ± 2.1
DMT (**1**)	7	11.8		60	5.8 ± 3.1	0 ± 0	48.2 ± 11.2	4.8 ± 2.1
NMT (**2**)	8	11.5	61		18.6 ± 3.4	0 ± 0	36.1 ± 9.5	0 ± 0
DMT/NMT	10	11.7	93	116	58.6 ± 6.8	7.7 ± 3.9	13.4 ± 5.5	13.4 ± 5.5

[a]Hard palate damage consisted of overlapping or missing bone. [b]The bones included in this column include sternebrae, the caudal, lumbar, sacral, and thoracic vertebrae, metacarpals, ribs, and parietal bones. Deformities include fewer than normal number of bones, greater than normal number of bones, or misaligned bones. [c]S- or C-shaped curvature of the spine in rat pups. [d]MeOH, methanol; NMT, *N*-methyltryptamine; DMT, *N,N*-dimethyltryptamine.

10% *M. tenuiflora* plant material or the components therefrom, extracted or synthesized, during gestation days 6−21. Rats were humanely euthanized (CO_2 asphyxiation) on gestation day 21, and pups were examined for cranial−facial defects including cleft palate and cleft lip and skeletal malformations. Nine different experimental diets were tested including *M. tenuiflora* leaf and seed material, crude methanol extracts of leaf and seed, crude alkaloid extracts of leaf and seed, and the purified alkaloids *N*-methyltryptamine (**2**) and *N,N*-dimethyltryptamine (**1**). The concentrations of **1** and **2** in each of the different diets were measured and reported in Table 1. The use of all animals was approved by the Institutional Animal Care and Use Committee (Utah State University, IACUC approval 1527) and conducted under veterinary supervision.

Preparation of Methanol and Crude Alkaloid Extracts. For an experimental group of 10 animals approximately 4 kg of feed was needed over a period of 15 days (gestation days 6−21). Therefore, for a 10% concentration in the feed, 400 g of *M. tenuiflora* leaf, seed, or extract from the equivalent amount of plant material was used in the formulation of the experimental group ration. Thus, 400 g of plant material was extracted with methanol (3 L) by Soxhlet extraction for 48 h. The methanol extracts were concentrated by rotoevaporation and transferred to a glass storage vessel by dilution in methanol at a concentration of 50 g of plant material equivalents per 100 mL. For preparation of the rations, 44 g of plant or the equivalent extract (88 mL) was combined with 360 g of rat chow (the methanol was removed by evaporation) and 36 g of corn starch and formulated as previously described.[3]

To obtain a crude base (alkaloid) fraction, an 88 mL aliquot of the methanol extract (equivalent to 44 g of plant material) was evaporated by rotoevaporation, the flask rinsed twice with 1% H_2SO_4 (50 and 25 mL), and the acid solution saved. The flask was then rinsed three times with dichloromethane (3 × 50 mL), the dichloromethane was subsequently extracted with 1% H_2SO_4 (2 × 100 mL), and all acid extracts were combined. The acid solution was applied to a Strata XC solid phase extraction column (5 g) (Phenomenex, Torrance, CA, USA) prerinsed with methanol (50 mL) and water (50 mL). The SPE column was then rinsed with water (150 mL) and methanol (150 mL). The crude alkaloid fraction was then eluted with ammoniated methanol (150 mL; methanol saturated with ammonia then diluted with methanol 1:10). The ammoniated methanol was removed by rotoevaporation and the residue then dissolved in methanol (50 mL) and stored at 2 °C until used in rat chow formulation. This procedure was repeated approximately nine times to provide the total alkaloid fraction to formulate the rat chow for one experimental group of 10 rats.

Synthesis of *N,N*-Dimethyltryptamine (1). *N,N*-Dimethyltryptamine (**1**) was prepared by synthesis from tryptamine using reductive amination,[4] with modifications, followed by cleanup with flash chromatography. Tryptamine (4.7 mmol, 750 mg) was combined with sodium triacetoxyborohydride (18.4 mmol, 3.9 g) and glacial acetic acid (9 mmol; 0.54 mL) in 40 mL of THF. The solution was stirred at room temperature, and 1.89 mL of formaldehyde (37%) (~23 mmol) was added dropwise; the reaction continued with stirring for 2 h. The reaction solution was transferred to a round-bottom flask and the THF solvent removed by rotoevaporation at 40 °C. The flask was then rinsed with 1 N HCl (2 × 40 mL) and the acid solution extracted with ethyl acetate (2 × 50 mL). The ethyl acetate extract was discarded, and the pH of the acid solution was increased to a pH value of 10 with a 20% KOH solution and the basic solution extracted with ethyl acetate (2 × 70 mL). The ethyl acetate extract was dried over anhydrous sodium sulfate and filtered, and the solvent was removed by rotoevaporation to give crude **1**. The crude reaction mixture was purified by flash chromatography over silica gel (50 g) (Biotage) using a solvent gradient of ethyl acetate (A) and methanol (B) at a flow rate of 50 mL/min. Solvent gradient was 5% B (4 min), 5−40% linear gradient (26 min), 40% B (5 min). Fractions (20 mL) were collected throughout the run, and fractions selected on the basis of the UV detection at 280 nm were further analyzed by HPLC-UV-MS. The purest fractions were combined to give the final product (**1**) (380 mg; 43% yield). The reaction was repeated as needed to supply sufficient product for rat chow formulations. The final product was characterized by NMR and MS analyses and purity estimated to be >95%. UV (acetonitrile/water with 0.5% acetic acid/0.05% TFA) 233, 273, 279 nm; MS (LC-APCI-MS) 189 (100%; MH^+), 144 (20), 130 (40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (br s, NH), 7.62 (d, 1H, J = 7.5 Hz, H-1), 7.34 (d, 1H, J = 8.2 Hz, H-4), 7.16 (dt, 1H, J = 7.9, 1.0 Hz, H-2 or H-3), 7.12 (dt, 1H, J = 7.9, 1.0 Hz, H-2 or H-3), 7.02 (br s, 1H, H-8), 2.95 (m, 2H, H-11), 2.64 (m, 2H, H-10), 2.34 (s, 6H, $N(CH_3)_2$) in agreement with previously reported ^{1}H NMR data.[1]

Quantitative Analysis of *N*-Methyl- and *N,N*-Dimethyltryptamine by LC-MS in Formulated Rat Chow Pellets. Samples of the formulated rat chow were analyzed for the concentrations of **1** and **2** by LC-MS. Six random pellets from each formulation were ground separately with a mortar and pestle. A 100 mg aliquot of each pellet was placed into a 15 mL screw-cap test tube and extracted with 4 mL of dichloromethane and 4 mL of 1% H_2SO_4 for 3 h by mechanical rotation. The samples were centrifuged and the upper aqueous layer removed and saved. The samples were then extracted a second time with 2 mL of 1% H_2SO_4 for 5 min and centrifuged and the upper aqueous layer removed and combined with first extraction. The total aqueous acid extract (~6 mL) was added to a prepared 200 mg Strata SCX SPE cartridge, prerinsed with 2 mL of methanol and 2 mL of water. The SPE column was rinsed with 2 mL of water and 2 mL of methanol. The analytes were eluted with 4 mL of an ammoniated methanol solution. The samples were evaporated to dryness under a flow of nitrogen at 60 °C and then dissolved in 1.0 mL of 50%

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methanol/0.1% TFA, and 5 μL injections were analyzed by LC-APCI-MS using a Betasil C18 column, 100 mm × 2.1 mm i.d. (Thermo Fisher, Waltham, MA, USA) and a linear gradient of acetonitrile (A) and water (0.05% TFA/0.5% acetic acid) (B). Flow rate was 0.300 mL/min with a gradient of 10% A (0−3 min), 10−70% A (3−10 min), and 70% A (10−15 min). Detection was by APCI-MS, and peak areas were measured from reconstructed ion chromatograms of the protonated molecules (*m*/*z* 175 and 189). Calibration curves were composed of a set of five standards prepared from the *N*-methyltryptamine and *N*,*N*-dimethyltryptamine at concentration levels of 40, 30, 20, 10, and 2 μg/mL.

RESULTS AND DISCUSSION

In the original work, Medeiros[3] clearly demonstrated the acceptable characteristics of the rat model for testing of teratogenic effects of *M. tenuiflora*. Data were presented showing no adverse effects on animals receiving the ration containing 10% *M. tenuiflora* seed as to feed consumption, weight gain, clinical signs of sickness, abnormal behavior, and organ weights, and no significant histological lesions were produced in the treated pregnant rats. However, significant fetal malformations were observed, clearly demonstrating the teratogenic effects of the *M. tenuiflora* seed. In the most recent experiments presented here, the *M. tenuiflora* seed formulation was repeated, but the investigation extended to leaf material and then to chemical extracts and purified compounds found in *M. tenuiflora* in an attempt to identify and further understand the active chemical components in the plant material responsible for the teratogenic effects.

As in the prior work, rats accepted the prepared diets and showed no significant differences between control and treated animals as to feed consumption, weight gain, and overall health (data not presented). For the analysis of the current experiments fetal malformations were assessed in three categories: (1) soft tissue cleft palate; (2) hard palate anomalies; and (3) skeletal deformities. The skeletal deformities were grouped together and included analysis of spinal column alignment (scoliosis) and development of the sternebrae, the caudal, lumbar, sacral, and thoracic vertebrae, metacarpals, ribs, and parietal bones.

From the current experiment groups, rat pups from mothers who received *M. tenuiflora* plant material, methanol extracts, alkaloid extracts, and purified *N*-methyltryptamines had a higher incidence of soft tissue cleft palate in all treatments (Table 1). The soft tissue cleft palate was the most consistent and significant observed deformity in pups from treated dams. This soft tissue cleft palate was characterized by incomplete closure or overlap of the tissue (Figure 1). Deformities of the hard palate were observed only in the combined dose of **1** and **2**.

Skeletal deformities, including missing, extra, or misaligned bones, were observed in all treatment groups to some degree (Table 1). An extreme example was a pup with missing vertebrae (Figure 2). Minor skeletal aberrations were noted in a few control animals and were considered in the treatment evaluations. In general, the skeletal deformities were highly random in type and frequency, but when added together were considerably higher in frequency in comparison to the control group.

Specifically noted was the frequency of observed scoliosis in Table 1 to highlight an observed difference in the results of the rat pups from mothers receiving the mixed *N*-methyl- and *N*,*N*-dimethyltryptamine treatments. The frequency of induced scoliosis was higher at 13.4 ± 5.5% in pups from the *N*-methyl- and *N*,*N*-dimethyltryptamine group compared to all other treatments (2.1 ± 1.7%).

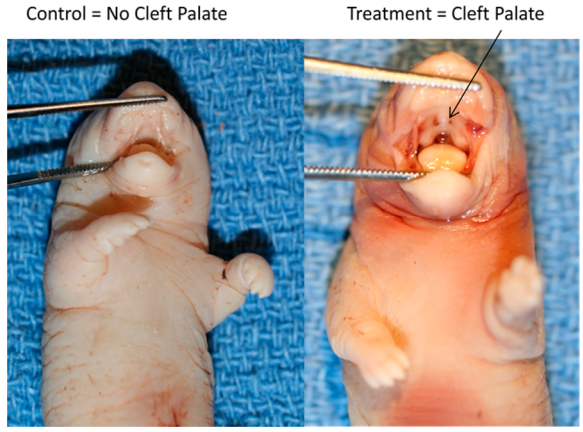

Figure 1. Observed induced soft-tissue cleft palate in a rat pup from a dam treated with *Mimosa tenuiflora* leaf and a control.

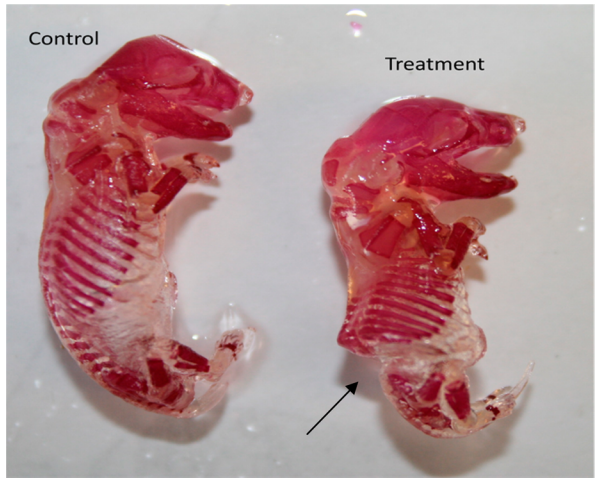

Figure 2. Observed skeletal malformation in a rat pup from a dam receiving a ration containing 10% *Mimosa tenuiflora* leaf and a control. Note complete lack of lower vertebrae.

In using the rat model, the teratogenic compounds were found to be extractable in methanol and not lost during further cation ion exchange solid phase procedures giving a teratogenic active crude base fraction. In previous work[5] the alkaloid content of *M. tenuiflora* was examined, and the major alkaloid fraction was found to be composed of *N*,*N*-dimethyltryptamine (**1**), 2-methyltetrahydro-β-carboline (**3**), and an unidentified alkaloid with a proposed molecular weight of 174. The unknown alkaloid has since been identified as *N*-methyltryptamine (**2**) (Figure 3). It was thus followed here with experimental diets using the two major alkaloids (**1** and **2**) in purified form, first in separate formulations and then in a combined (**1** and **2**) fortified ration.

Dams receiving **1** or **2** only rations resulted in pups with a slightly lower frequency of induced cleft palate compared to the original plant material or even the methanol or crude base extracts; however, skeletal deformities were of similar frequency (Table 1). In the final experiment using a combined **1** and **2** fortified ration, an increase in cleft palate and skeletal deformities was expected. Cleft palate induction frequency indeed increased (58.6%), and in a few cases there was even observed hard palate damage. An unexpected finding was the

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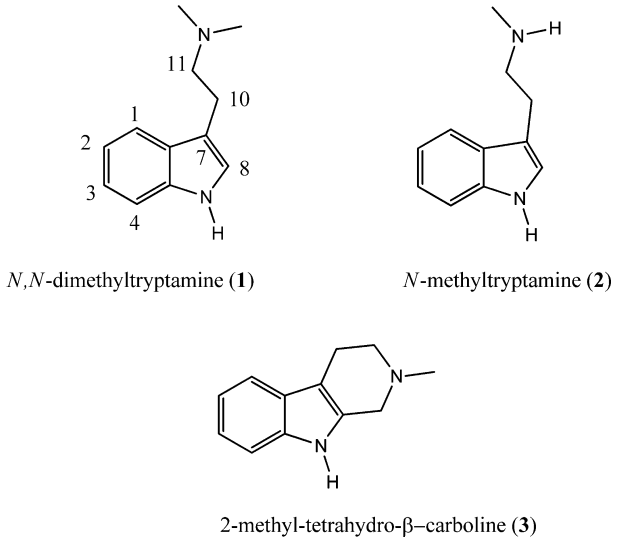

Figure 3. Structures of alkaloids in *Mimosa tenuiflora*.

total lack of skeletal deformities such as missing or misaligned or extra bones in this series of pups, although the frequency of induced scoliosis increased. There are no prior reports of any teratogenic effects of **1** or **2**. They are best known for their psychoactive properties.[6−8] There is currently insufficient information to allow speculation on the possible teratogenic mechanism of action at this time.

In summary, the use of the pregnant rat model for the testing of crude chemical extracts, semipurified extracts, and even purified compounds for teratogenic effects of *M. tenuiflora* seems plausible. The results confirmed that the active principles could be extracted from the plant with methanol and were carried through an ion exchange procedure to what would be considered a crude alkaloid fraction. However, other compounds of basic characteristics, for example, basic amino acids, would also be present in this crude alkaloid fraction. Both **1** and **2** were found to be teratogenic in the rat model as they induced cleft palates and skeletal malformations. Future work should include confirmation of the teratogenic activity of *N*-methyl- and *N,N*-dimethyltryptamine in a small ruminant model.

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Notes

The authors declare no competing financial interest.

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