Aphrodisiac Properties Of *Turnera Diffusa*

Liza (Jodarski) Helmrick and Charity Reiser

Faculty Sponsors: Margaret A. Maher, Department of Biology and Aaron Monte, Department of Chemistry

ABSTRACT

The purpose of this investigation was to determine if the tea plant *Turnera diffusa*, more commonly known as damiana, had a measurable effect on sexual behaviors in mice. Injections of damiana suspended in peanut oil (for test groups), or peanut oil alone (for control groups), were given to male and female mice. After two weeks, male mice were introduced to female mice following induction of estrus. Five sexual behaviors (number of mounts, intromissions, ejaculations, latency period, and lordosis) were monitored independently by two investigators in real time or using videotape. The results showed that the number of mounts and intromissions for the test mice were significantly higher than that of the control mice. Results from ejaculations, latency period, and lordosis were not statistically conclusive. No litters were obtained from test or control copulated females, suggesting that the peanut oil may have altered fertility. Accordingly, damiana does appear to exhibit some aphrodisiac properties in mice. Further studies may be done to determine the differential effects of damiana on male versus female mice.

INTRODUCTION

Aphrodisiacs have been of great interest to humans since the beginning of time. An aphrodisiac is defined as an agent (food or drug) that arouses sexual desire. Many natural substances have historically been known as aphrodisiacs in Africa and Europe, such as yohimbine and the mandrake plant, as well as ground rhinoceros horn in the Chinese culture and "Spanish fly" which is actually toxic (Ang et al., 1997; Rosen et al., 1993; Evans, 1969). Even in today's culture there are certain foods that are used as aphrodisiacs, including strawberries and raw oysters. Although these natural items are touted as aphrodisiacs, there is little scientific evidence supporting those assertions.

In a recent study conducted in the Boston area, 52% of men between the ages of 40 and 70 reported some degree of erectile dysfunction (Marshall, 1998). Enhanced sexual behavior may provide increased relationship satisfaction and self-esteem in humans (Montorsi et al., 1995). Therefore, the study of aphrodisiacs is important because they may provide a means to treat the psychological components of sexual dysfunction as opposed to the current treatments, surgical implants and injection therapy, which only treat the mechanical component (Rosen et al., 1993).

Turnera diffusa, commonly known as damiana, has been used in folk medicine for many years, especially as an aphrodisiac. The plant grows in Brazil, Bolivia, Mexico, the West Indies, and California (Claus, 1956; Tyler et. al., 1988). This shrubby perennial can grow up to 6 feet tall and has smooth, pale green leaves and small yellow flowers. The leaf of the

plant is harvested during the summer and is used in teas and as a flavoring for liqueurs. Damiana teas were prescribed in many situations to help "heal" certain ailments. The teas served as treatment for mild depression, physical weakness, hangover, dyspepsia, and nervous disorders. They are also thought to stimulate appetite, digestion, and metabolism (Spencer et al., 1981). Although little scientific research has been done on damiana, contradictory reports of its effects as a sexual stimulant can be found in current literature, on the internet, and in many magazines (Marshall, 1998). The purpose of this investigation was to determine if damiana does indeed exhibit aphrodisiac properties in mice.

METHOD

Damiana Solution

A Soxhlet extraction apparatus was used to obtain a crude extract from damiana leaves. One hundred fifty grams of dried damiana leaves were packed into a thimble made of porous cellulose material, and the round bottom flask was filled with 160 mL of 95% ethanol and heated to a boil. After 24 hours, the damiana leaves were removed and a fresh 150 g sample of damiana leaves was repacked into the thimble, and the volume was refilled to the original 160 mL of solution. After 24 hours, the solution was poured into evaporating dishes. Two days later the same procedure was repeated using 120 g of damiana leaves. The two extracts were combined in the evaporating dishes and left under the hood for four days. The evaporating dishes were placed on top of an oven set at 120 °C to allow for the rest of the solvent and water to be evaporated. The extract was removed from the evaporating dishes by scraping with a spatula into a 500 mL round bottom flask. Enough 100% ethanol was used to remove the last traces of the extract from the dishes. The solvent was then removed on a rotary evaporator. When most of the solvent had evaporated, the solution was placed under high vacuum for approximately 24 hours. The flask was weighed, purged with nitrogen gas, and wrapped in aluminum foil to keep as much light and air away from the extract as possible. The weight of the extract was 107.0 g from 420 g of dried damiana leaves. A solution of this extract and peanut oil was prepared. The solution was stirred for two hours and briefly sonicated to form a homogeneous suspension. It remained in this form for two weeks and was sterilized using a 25 mm syringe filter with 0.2 micron multiple ester cellulose membrane to remove particulate matter. The final solution was a pea green color.

Pharmacological Bioassay

Eight groups of mice, four test and four control, were used in the bioassay. Forty NIH Swiss mice were used, weighing 21-24 g when received. Each of the eight groups contained five mice, four females and one male. All mice received doses that contained 10 mg of damiana in 0.3 mL peanut oil (or 0.3 mL peanut oil for control) every other day for two weeks for a total of eight doses. Each week, one control group and one test group would begin dosing. The following week the next would begin, etc. Prior to dosing, the females were numbered using ear punches, and all mice were weighed before the first dose and before the last dose. The doses were administered by subcutaneous injections, using 26 gauge, 1/2" needles with 1 cc tuberculin syringes. Three days prior to the final administration of damiana, female mice received an injection of β-estradiol-3-benzoate (10μg in 0.05 peanut oil, subcutaneous). On the last day of administration of damiana, female mice received an injection of progesterone (500 μg in 0.05 ml peanut oil, subcutaneous). The hormone injections were administered to ensure the females were in estrous, the time when they are most receptive to fertilization (Morales et al, 1996; Guttman et al., 1975). After two weeks of administrations, the test

males were paired with test females, and the control males were paired with control females for one-hour observation periods. Five behaviors were monitored as described in Table 1: mounts, intromissions, ejaculations, latency period, and lordosis (McGill, 1965).

Table 1: Definitions of observable sexual behaviors in mice.

BEHAVIOR	DEFINITION
Mount	Male approaches a female and assumes a copulatory position
Intromission	Male penis penetrates into female vagina, observed by decreased rate of thrusting
Ejaculation	Observed by an increased rate of thrusting after intromission, followed by the male cleaning himself
Latency Pd.	Time elapsed from introduction of male with females to first mount
Lordosis	Female firmly raises hind quarters and tail to accept male advances

The mice were maintained in a constant environment with a 12:12 hr light:dark cycle. Observations were conducted between 2 to 3 hours after the onset of the dark half of the cycle under a red light, as this is the time when these rodents are most active (Morales et al., 1996). The male mice were marked using a permanent marker with a large black X on their back for easy identification. Two independent observers, blind to the conditions (test vs. control), scored the behaviors. One watched the activity live and the other watched videotapes. The mice were euthanized by carbon dioxide inhalation followed by cervical dislocation to ensure death. All procedures in this study involving animals were approved by the University of Wisconsin-La Crosse Institutional Animal Care and Use Committee.

RESULTS

To test the directional hypothesis that damiana would have aphrodisiac effects, the significant differences between mean number of events for each behavior were assessed by SigmaStat® 2.0 using one tail two-sample t-tests. The results are shown in Table 2. Damiana significantly increased male mounts and intromissions. There were no significant differences among groups for male ejaculations or latency periods or for female lordosis events.

Table 2: Effects of damiana versus control (peanut oil only) injections on five sexual behaviors in mice.

GROUP	MOUNTS	INTROMISSIONS	EJACULATIONS	LATENCY PERIOD	LORDOSIS
Control mice	35.0 ± 7.7	4.8 ± 2.4	0.8 ± 0.5	725.8 ± 151.7	3.4 ± 1.5
Damiana mice	56.6 ± 6.8*	11.4 ± 1.7*	2.1 ± 0.9	1027.3 ± 192.6	6.3 ± 2.3

Values are depicted as group means ± standard errors

*Significant differences from control are indicated with an asterisk, $p<0.05$ as assessed by one-tail t-test.

DISCUSSION

The results of this study suggest that injected damiana extract may have aphrodisiac properties in male mice placed in an optimal environment for sexual behavior, with supposedly receptive females (Luttge and Hall, 1976; Guttman et al., 1975; McGill, 1965). Damiana did not appear to exert significant aphrodisiac action on female mice. The small number of groups (n=4 per condition) observed limited the power of the statistical methods. There was a large degree of variation within groups. This may be, in part, due to variable distribution of both control and damiana injections among individuals. It was observed that sometimes the fluid seemed to dissipate from the site of the injection and at other times it did not.

Interestingly, none of the females in the control or damiana groups gave birth to pups, even though some ejaculations occurred. It is therefore suspected that the frequent injections of the peanut oil vehicle may have had physiological effects on sex hormone status, fertility, and/or reproductive behavior in both groups. Unfortunately, sperm counts and vaginal smears were not performed to assess reproductive capacity in these mice. While peanut oil is a standard vehicle for injection of sex hormones in animal studies, the amount of oil injected is historically much less than was required in this study to deliver the desired amount of damiana.

This study was designed to allow damiana to exert the greatest effect possible so both males and females in the damiana group were treated. However, it might be useful to study the effects of damiana in males with untreated females and in females with untreated males. Another useful alteration of this study may involve use of a more concentrated damiana solution that requires less vehicle for delivery. For optimal assessment of damiana's potential use as an aphrodisiac to ameliorate sexual dysfunction, a long-term consumption study should be performed in a reasonable animal model prior to testing in humans.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the contributions of Amy Cooper, the UW-L College of Science and Allied Health Animal Facility Manager, for assistance with the mice, and Wayne Abler and The UW-L Educational Technology Center for assistance with videotaping. In addition, special thanks to the UW-L Undergraduate Research Grants Program for the funding of this project.

REFERENCES

Ang HH, Chan KL, Gan EK, Yuen KH: 1997. Enhancement of sexual motivation in sexually naive male mice by *Eurycoma Longifolia*. **International Journal of Pharmacology** 35:144-146.

Claus EP: 1956. *Pharmacognosy*, **3rd Edition**, (Lea & Feberger, Philadelphia, PA) 387.

Evans WO: 1969. Chemical Aphrodisiacs. **Psychopharmacology Bulletin** 5(2):11.

Guttman R, Lieblich I, Gross R: 1975. Behavioral correlates of estrous stages in laboratory mice. **Behavioral Biology** 13:127-132.

Luttge WG, Hall NR: 1976. Interactions of progesterone and dihydroprogesterone with dihydrotestosterone on estrogen activated sexual receptivity in female mice. **Hormones and Behavior** 7:253-257.

Marshall MA.: 1998. A dose of desire. **Mademoiselle** Nov: 90-92.

McGill TE: (1965) Studies of the sexual behavior of male laboratory mice: effects of genotype, recovery of sex drive and theory. In: Animal Behavior (Holt, Rinehart, & Winston, New York, NY) 76-88.

Montorsi F, Guazzoni G, Rigatti P, Pozza, G: 1995. Pharmacological management of erectile dysfunction. **Drugs** 50(3):465-479.

Morales J, Larraide C, Arteaga M, Govezensky T, Romano MC, Morali G: 1996. Inhibition of sexual behavior in male mice infected with *Taenia Crassiceps Cysticerci*. **American Society of Parasitologists** 82:689-693.

Rosen, RC, Ashton AK: 1993. Prosexual drugs: empirical status of the "new aphrodisiacs." *Archives of Sexual Behavior* 22 (6): 521-543.

Spencer KC, Siegler DS: 1981. Tetraphyllin B from *Turnera Diffusa*. **Planta Medica** 43:175-178.

Tyler VE, Brady LR, Robbers JE: 1988. **Pharmacognosy, 9th Edition**, (Lea & Feberger, Philadelphia, PA) pp 469.