Cedrela odorata acts as a Contraceptive in Adult Female Mice

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Abstract
The researchers wanted to find a medicinal plant that could be used in the pharmaceutical world as a contraceptive due to women looking for a safer and more effective one. According to Rainforest Remedies by Dr. Michael Balick and Rosita Arvigo (1998), Mayan women would make Cedrela odorata bark into tea and drink it three times a day for three days in order to cause sterility for one cycle. Hence, the researchers felt that this needed to be researched further to determine if it could become the contraceptive women were looking for. To assess the efficacy of Cedrela odorata bark made into tea at the dose of 0.15 milliliters, (CD-1) female mice were administered tea made from the bark three times a day for three consecutive days, followed by copulation with male mice. The tea intercepted 92% of the pregnancies in female mice among the three experimental groups when administered orally for three days with three doses of 0.15 milliliters. All of the control group mice became pregnant when administered orally for three days with three doses of 0.15 milliliters of distilled water. No mortality and changes in the behavior were observed from pre-dose days to the postcoitum days in all the control and experimental groups. The two-tailed P value ≤ 0.0001 compared the number of pregnancies of the six experimental groups to the number of pregnancies of the two control groups. Findings demonstrated that there was a significant contraceptive efficacy of the Cedrela odorata bark made into tea at the dose of 0.15 milliliters. More research is needed to determine the length of sterility and the mechanism of the bark. This research will help meet the increasing need for population control due to no clear improvement in the effectiveness in contraceptives between 1995 and 2002 (Kost et al. 2008). Being of plant origin would allow for a relatively cheap and effective contraceptive that could become widely available and accepted.

Introduction
There has been an increase in medicinal plants integrating into human society to combat various diseases, ranging from skin infection to gastrointestinal problems (Gbotolorun et al. 2008). Previous literature has documented Cedrela odorata as being one of the many plants used in tribal and herbal medicines. These plants have been used for centuries by many tribes throughout the world. That is why researchers are looking into plants as a possible solution to finding a more effective contraceptive that women would use. Natural plant substances possessing mild inherent estrogenic and antiestrogenic properties offer themselves as effective non-conventional sources of contraception with less deleterious side-effects (Yadav and Jain 1999). This is why continuous efforts are being made to use natural plants to develop antifertility products.

Cedrela odorata, commonly known as Spanish Cedar, is native to the tropical region of South America. The durability and resistance to insects of this deciduous tree has made it most sought-after for the manufacturing of veneer and furniture (USDA 2007). Growing between 60 to 90 feet with a 3.5 foot diameter, the gray bark is deeply grooved with two thirds of the tree being branchless (Griffin 2003). Dr. Rhys Thomas, Director of Fayette Environmental Service, and co-workers (2003) determined that benzaldehyde is the active ingredient in the bark through Mass Spectrometry. Benzaldehyde only becomes active when the tea is warm (Griffin 2003).

Survey of literature revealed that Cedrela odorata has not been researched much when it comes to the medicinal aspects of the tree. However, there is documentation that the tree has been used in tribal and herbal medicine and can help with various health problems. The bark is of most use usually being infused or tinctured. An infusion of its bark is used as a remedy for diarrhea, fever, vomiting, hemorrhages, dyspepsia, bronchitis, and indigestion (USDA 2007). The decoction of the bark is used against malaria and fever in Africa (Kipassa et al. 2008). The bark was distinguished by having the highest percentage of respondents reporting its uses, as well as the most diverse applications, and was cited as a treatment for kidney problems, diabetes, rashes, inflammations, and headaches (Mutchnick and McCarthy 1997).

Along with the treatments mentioned, there are many more uses depending on the location or tribal area. In the Brazilian Amazon the bark and leaves are added to baths for body pains, colds, flu, and fever, while in the Peruvian Amazon a bark infusion is used for diarrhea, urinary problems, and ear infections (Taylor 2005).
There is very little literature confirming these. Hence, more research needs to be done dealing with *Cedrela odorata* and its health benefits.

One benefit of *Cedrela odorata* that needs to be investigated is its antifertility activity for women. According to Dr. Michael Balick and Rosita Arvigo (1998) in Rainforest Remedies, the Mayan women would drink three cups of tea three times a day for three days. The women would boil a 10-inch piece of bark in 3 cups of water and make the tea fresh each day. For up to one cycle, the women would be sterile (Arvigo and Balick 1998). But so far there has been no published antifertility activity research carried out on *Cedrela odorata*. Therefore, the researchers were interested in repeating the steps of the Mayan women when making the tea at a dose equivalent for female mice.

They wanted to eliminate the aspects of the teas’ possibility in causing side effects to show that the tea had a great chance of becoming a contraceptive. Comparison of the three dosage stages would determine if there was any abnormal behavior among the mice caused by the tea. A change in their normal home cage behavior due to the tea would be if the mice showed any abnormal movements or were awake more during or after the dosing period. If during or after the dosage the mice became more vocal, froze, sat, or rattled their tail, their normal removal reactions would be considered disturbed by the tea. If the mice became more tense or quiet with no resistance during or after the dosage, it would show that the tea was affecting their normal handling reaction.

Developing antifertility products has been continuously active in research. The total estimate of 122.7 million women with unmet need represents a substantial and continuing challenge for agencies and governments concerned with ensuring access to contraceptives (Ross and Winfrey 2002). Little research has been done on the antifertility activity of *Cedrela odorata*. So the researchers wanted to answer the question, Will *Cedrela odorata* act as a contraceptive in the mice? The overall aim of this study was to develop a female contraceptive that would be accessible, cheap, and effective for the majority of women. The specific objectives were: investigation of the *Cedrela odorata* bark’s effects on the estrous cycle and ovulation, and determination of the possible side effects of the tea in female mice. The researchers believed that at the dose of 0.15 milliliters, *Cedrela odorata* tea will act as a contraceptive.

### Materials and Methods

#### Plant collection and preparation of tea

One kilogram of *Cedrela odorata* was donated by the USDA-ARS, SHRS, National Germplasm Repository in Miami, Florida. A 3-1/2 inch piece of bark with an inch wide was boiled in 237 milliliters of distilled water for 10 minutes and then filtered by placing filter paper in a filter on top of an Erlenmeyer flask. These figures were determined by downsizing the actual amount of bark and water that the Mayan used by three due to the equivalent amount for female mice being much lower. It was allowed to cool to 27°C before giving to any mouse. A new mixture was made fresh each day early in the morning and was kept at room temperature throughout the day.

#### Animals

ICR (CD-1) mice weighing on average 33.6 grams were for the antifertility experiments. The mice were weighed by putting a small basket on a scale, the scale was then tared, the mice were then placed in the basket and the weight recorded. The weights in grams were then added up and divided by sixteen, the total number of female mice, to get the average overall weight of the mice. This was done by Addison Labs in Fayette, MO (who were the ones that provided the mice).

These albino mice are derived from Charles River Laboratories in Wilmington, Massachusetts and are the most widely used outbred mice with excellent reproductive and maternal characteristics. On average, they produce 11.5 pups per litter (Charles River Laboratories 2007).

All the mice were maintained under standard laboratory conditions (temperature 22 +/- 3°C and 12-hour light/dark cycle) with food and water ad libitum. The lights were turned on at 7:30 a.m. and turned off at 7:30 p.m. Fresh water was given at 7:30 a.m. daily along with the food being checked at the same time. Pine shaved bedding was changed twice a week.

After random selection sixteen mice were placed into eight cages with two female mice per cage. The mice were kept under hoods in the lab with four cages per hood. The eight cages of female mice were labeled as:

1. **Hood 1:**
   a. Control Group 1
   b. Control Group 2
   c. Experimental Group 1A
   d. Experimental Group 1B

2. **Hood 2:**
   a. Experimental Group 2A
   b. Experimental Group 2B
   c. Experimental Group 3A
d. Experimental Group 3B
All mice were returned to Addison Labs for disposition. All procedures involving animals in this study was approved by the Central Methodist University Institutional Review Board.

Antifertility Study
There were three phases for this study:

1. Phase One: Pre-Dose Group
   a. This phase was considered the two weeks before the mice were given the tea.

2. Phase Two: Dose Group
   a. This phase was considered the three days that the same mice received the 0.15 milliliters of tea.

3. Phase Three: Post Dose Group
   a. This phase was considered the twenty-one days after the mice were given the tea plus the days the male mice were added.

Each phase included an assessment of all the female mice. The same female mice were used for each phase.

Female mice were observed daily at intervals of ten minutes in the morning and ten minutes in the evening for two weeks prior to testing in order to determine their normal behavior to help with assessment of possible side effects, and to accommodate them to the environment.

Each female mouse in the six experimental groups was administered 0.15 milliliters of tea by an intubation tube in a syringe, while the four mice in the two control groups each received 0.15 milliliters of distilled water by an intubation tube in a syringe.

One cup of tea in a female human is equivalent to 0.15 milliliters in a mouse. The dosage was determined based on Allometric scaling using the average weight of the mice (33.6 grams). The dosage amount for the experiment was determined using the dose calculator on the FDA’s website to scale the metabolism of a mouse to a female human (2007). The average Mayan female height is 142.2 centimeters and the average weight is 50 kilograms (Disabled World 2008). After plugging in the average Mayan female’s height and weight and the dosage value of 4535 mg/kg, the dose equivalent was 152.38 mg, which is 0.15 milliliters. The dosage value of 4535 mg/kg was determined by taking one cup, which is 226796.19 mg, divided by their average Mayan weight.

One by one the female mice were lifted out of their cage by grasping their tail. The mouse was then allowed to grasp the wire cage top to allow one of the researchers to restrain the mouse by pinching the loose skin around the neck and shoulder area with the thumb and forefinger. The tail was then held down by the pinky on the same hand to allow for further restraint. The mouse was turned over with its stomach facing up, while the other researcher stuck the syringe with the intubation tube down into the female mouse. Then the mouse was placed into a second cage that only contained bedding and no other mice. Once the second mouse in the group was given tea, the other mouse was returned to the cage. Groups were done one at a time with the experimental groups done first. After injecting all of the experimental mice, the syringe was filled with distilled water and sprayed into a sink for cleansing.

After three days, the female mice were given one day to allow for the tea to take affect. One male mouse was then placed in each of the eight groups and left there until a vaginal plug was viewed. Each female mouse was checked daily for a vaginal plug. To determine if copulation has occurred, observation must be done early in the morning for the vaginal plug (Griffin 2003). The males were then removed and the females were observed for signs of pregnancy and side effects. They were observed for a month due to their gestation period being 19-21 days.

The mice in both the control and experimental groups were marked with a sharpie marker to distinguish them individually. One mouse in each A group was marked with four slashes on the tail and the other mouse was marked with a triangle on its tail. In each B group, one mouse was marked with two slashes on its tail and the other mouse was marked with an A on the tails.

Previous research done by the same researchers in the fall confirmed the mice to be fertile. The same female mice were run through the exact experiment in the fall except with a dosage of 0.02 milliliters and bark that was not confirmed as Cedrela odorata. Along with this experiment, there was only one other research of antifertility using Cedrela odorata that was found. This research was done by a student, Megan Griffin (2003) a graduate of Chillicothe High School, of the professor that was the overseer of this current project. The data showed Cedrela odorata to be an effective antifertility. Neither one
of these studies mentioned are published, but were available to the researchers.

Behavioral Study
An individual study functional observation was performed on the CD1 (ICR) female mice in the control and experimental groups to determine if there were any side effects. They were observed in four phases.

1. Phase One: Pre-dose Phase
   a. This phase consisted of days 1-12 before dosage.

2. Phase Two: Dosage Phase
   a. This phase was the three days that the mice were given the tea.

3. Phase Three: Copulation Phase
   a. This phase included the day before adding the male mice and the days with the male mice.

4. Phase Four: Post Dose Phase
   a. This phase was days 1-21 post-coital.

Each female mouse in all eight groups was observed in the morning when the lights were turned on. There were six categories to help determine change in behavior. The assessment was done with the following categories and subcategories:

1. Home Cage Behavior
   a. Sleeping
   b. Awake, Immobile
   c. Normal Movement
   d. Unusual Posture
   e. Unusual Behavior

2. Alterations
   a. None
   b. Stereotyped
   c. Bizarre
   d. Limb Twitches/Tremor
   e. Whole Body Tremor/Spasm
   f. Unusual Posture

3. Removal Reaction
   a. Sit
   b. Vocalization
   c. Runs
   d. Freezes
   e. Tail/Throat Rattles

4. Handling Reaction
   a. Quiet, No Resistance
   b. Vocal
   c. Tense
   d. Squirming

5. Level of Arousal
   a. Stuporous
   b. Sluggish
   c. Normal
   d. Sudden Darting
   e. Freezing, Vocal

6. Appearance
   a. Clean and Groomed
   b. Unkept
   c. Urine and/or Fecal Stain

During phases 2-4, the mice were assessed only once a day for ten minutes in the morning before dosage was given. Only the home cage behavior, removal reaction, and handling reaction were graphed. Each mouse's behavior was recorded under their group's column for each category. The pre-dose, dose, and post dose days were charted. When charted, the third and fourth phases were combined as the post dose days.

The comparison between the dosage stages helped in determining whether or not the tea caused any abnormal behavior among the mice. If the mice showed any abnormal movements or were awake more during or after the dosing period it would then show that the tea had changed their normal home cage behavior. If the mice became more vocal, froze, sat, or rattled their tail during or after the dosage, the tea would then have been interacting with their normal removal reactions. If the mice became more tense or quiet with no resistance during or after the dosage, it would show that the tea was affecting their normal handling reaction. By eliminating the aspects of the teas’ possibility in causing side effects, the researchers are able to show that the tea has a great chance of becoming a contraceptive.

A bar graph was used to show an increase or decrease in their behavioral actions for each category. The control and experimental groups were then compared to each other in one bar graph for each pre-dose, dose, and post dose phases. A bar graph was then developed to show all three phases together.

Statistical Analysis
Results in graphs were expressed as means. Excel was used to average the number of mice in each behavioral subcategory for each control and experimental group. The data was analyzed by Student’s t-test comparing the number of pregnancies among the six experimental groups to the number of pregnancies among the two control groups. The significance level considered was at or below p < 0.05.

Results
Antifertility Study

The Cedrela odorata bark made into tea intercepted pregnancy in an average of 92% of the
female mice among the three experimental groups (Figure 1). All four of the control group mice, which were given 0.15 milliliters of distilled water, became pregnant. The appearance of the plug suggested that mating had occurred in all four female mice in the control and all twelve female mice in the experimental groups.

The average litter was the same among the four female mice in the two control groups (7 pups). There was no average litter for the three experimental groups due to only one female mouse in experimental group 1 becoming pregnant (4 pups) (Figure 2).

During the pre-dose days, all experimental and control groups slept about the same amount with experimental groups 2 and 3 being awake the most. These two groups had on average more mice that were moving around when observed.

When removed, all of the control and experimental groups ran, with experimental group 3 having a mouse that sat once in a while. Experimental group 2 was the most vocal when removed. This group on average had more mice squeak while the other groups had mice that did not make any noise when removed on average. Handling
caused all the mice to be squirmy and vocal with experimental group 1 not being vocal at all. A mouse was considered squirmy if they tried to get loose while in the researchers’ hands (Figures 3-5).

In the dose days, the control group seemed to sleep the most, while the experimental group 1 was awake the most. All of the mice ran when removed. During the handling, all of the experimental and control groups squirmed and were vocal. However, the control group was not vocal and experimental group 3 was the most vocal (Figures 3-5).

During the post dose days, the control group slept the most, while the experimental groups were more active. When removed, all of the groups ran and were vocal. However, the control group was not vocal and experimental group 3 was the most vocal. Handling caused all groups to squirm and all to be vocal except the control group (Figures 4-5).

When compared, there were no significant differences among the actions during the experiment (Figures 4-5). Within the home cage behavior, the control was more awake during the dosage days, while the experimental groups were consistent (Figure 4). When removed, all groups ran consistently, while experimental group 3 sat once during the pre-dose days. The experimental groups were vocal with groups 1 and 3 being more vocal during post dose and group 2 being more vocal during the pre-dose (Figure 5). All groups were squirmy during the handling of the mice, with the control group being more vocal during the pre-dose days, however the experimental groups were consistently vocal (Figure 5).

A mouse was considered awake if it was moving around with normal movement. One that was curled up and eyes closed was asleep. Those that ran as the mouse was being taken out of the cage was considered running under the removal reaction. There were also mice that would sometimes sit still when removed. To be considered squirmy when handled was if the mouse tried to escape. The mice were considered vocal if they squeaked during removal or handling.

All of the results were averages in each individual group. Meaning that the same mouse was not necessarily the vocal one every time. Some of the mice were never vocal or never ran, while others were. Overall the averages proved to show no significant differences throughout the entire study.

**Statistical Analysis**

The student t test results were significant. Using the GraphPad Software’s QuickCalcs the two-tailed P value was found to be less than 0.0001, making the research extremely statistically significant (2002). This compared the number of pregnancies of the six experimental groups to the number of pregnancies of the two control groups. There was a 95%

<table>
<thead>
<tr>
<th>Group</th>
<th>Group One</th>
<th>Group Two</th>
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<tr>
<td>Mean</td>
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<tr>
<td>SEM</td>
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<td>0.00</td>
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<tr>
<td>N</td>
<td>32</td>
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Table I. Statistical results showing the mean, standard deviation, standard error of the mean, and normal distribution from the unpaired t test. The two-tailed P value was ≤ 0.0001 being considered extremely significant. The number of pregnancies in the six experimental groups was compared to the number of pregnancies in the two control groups for the statistical analysis.

confidence interval of a difference from -7.93 to -5.40. The t value was 11.2815 with 14 degrees of freedom and a standard error of difference of 0.591. There was a review of the data with Group One being the experimental groups and Group Two being the control groups (Table 1).

**Discussion**

Our study demonstrated that *Cedrela odorata* bark made into tea at the dose of 0.15 milliliters could
be an effective contraceptive. There were no major differences among the number of pups in a litter between the two control groups and only one mouse in the experimental groups became pregnant with a litter of four.

The tea proved to have no side effects on the mice mating, their cleanliness, and their sleeping habits. The t test showed our research to be extremely statistically significant with the p value being less than 0.0001. The two-tailed P value ≤ 0.0001 compared the number of pregnancies of the six experimental groups to the number of pregnancies of the two control groups.

Our previous semester’s research did show some benefits to it. It proved that all of our male and female mice were fertile. It also showed that there are several variables involved in this research. The bark used last semester was from TropiLab Inc. in Florida and was in mulch form. This time we used certified bark from the USDA National Germplasm Repository in Miami, Florida. The dosage was changed from 0.02 milliliters to 0.15 milliliters due to the average weight and height being Mayan the second time. The way the tea was prepared changed along with the previous changes. While noting the variables, there still was an indication of some interaction of the Jesupra odorata in the menstruation cycles of the mice.

Sources of error that could have hampered the interpretation of the data would include the fact that the data was averaged out. With averages the researchers had to take into consideration that not all of the mice in one group was vocal or ran. One mouse could have been the one that was vocal every time. The researchers had to be very observant and record the mouse in the column that represented its group immediately after handling that particular mouse. When it came to being vocal when handled the researchers recorded if they were vocal before the intubation tube with the syringe was put in the mice.

The schedule of the study was based on the mice’s cycle. In a laboratory mouse, the normal estrus cycle is 4 to 6 days long (Silver 1995). The five stages involved in the cycle are diestrus, proestrus, mestrus 1, and mestrus 2 (Champlin 1973). Their gestation period lasts 19 to 21 days and ovulation occurs 3 to 5 hours after a dark cycle. Breeding most likely occurs late at night due to the mice becoming more active closer to midnight.

There are no studies that have explored the mechanism of Ciderola odorata’s plant extract that may be employing to induce the cycle of sterility. The researchers have not confirmed that benzaldehyde is the main plant extract causing the infertility for one cycle. Hence, further investigation on the plant extracts need to be done. The researchers hypothesize that the Ciderola odorata extract alters the estrous cycle causing a reduction in the frequency of ovulation and impairment in fertility.

Further studies need to be carried out to determine the effect and content of benzaldehyde in the species that we used. This was determined based on the fact that there were no significant changes in the mice’s behavior for all the experimental and control groups throughout the entire study. The behavioral analysis was done in averages meaning that a certain group on average had so many mice vocal or sleeping. Due to insufficient research on Ciderola odorata bark, the effects of it on humans that have come in contact with it is unknown to researchers. As mentioned before it has been used for years in herbal and tribal medicine.

Overall this study is helping in identifying a new antifertility drug that could be effective, safe, and socially acceptable for human females. It would be widely available due to it being native to the tropical region of America and its introduction into cultivation in Africa and several South-East Asian and Pacific countries (Kipassa et al. 2008). More trials are needed to prove its safety before trials can begin on women. This would be an alternative to the traditional contraceptives of today. This could help prevent abortions from unintended pregnancies found in today’s society. Due to the dosage being done within three days for one cycle, women would be more willing to use this as a contraceptive choice. This would allow women to take something for three days and then not have to worry about it until their next cycle. As a natural substance this contraceptive would give women a possible chance of fewer side effects and possibly be less costly. It is probable that an oral herbal contraceptive would allow couples to control their fertility, which might in turn likely increase the number of couples practicing family planning (Gbotolorun et al. 2008).

Administration of Ciderola odorata bark made into tea causes sterility for at least one cycle in female ICR (CD-1) mice and has the potential of an ideal antifertility agent. This justifies further investigation of the detailed antifertility activities of Ciderola odorata along with identification of its active components.

Acknowledgments
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