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# Efficacy of dl-tetrahydropalmatine (component of *Corydalis yanhusou* extract) as a Sedative on Mice

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## ABSTRACT

Anesthetics are substances that produce sedative effects often experienced as low pain perception or sedation. Presently there is a rise in popularity of holistic medicine in the United States, partly because of the associated negative effects with many synthetic compounds. Some common anesthetics used for small animals during procedures include acepromazine, isoflurane, and thiopental. All the above synthetic compounds show negative side effects. In this experiment, tetrahydropalmatine, a compound isolated from corydalis extract was tested for its efficacy as a sedative in mice through intraperitoneal injection. Tetrahydropalmatine is found in two different stereoisomeric forms (dextro and levo), but for this experiment a mixture of d/l isomers was used. The experiment consisted of four experimental groups of mice, of which three received treatment and the fourth was the control group. Sedation was monitored by observing their motor control abilities following the administration of the compound. The data was statistically analyzed using 'repeated measures' to make comparisons between doses and sedation times. From the results we conclude that the differences of sedation times between the three treatment groups were statistically significant as shown by the p-value of $< 0.001$.

Keywords: *Anesthetics, Complementary and Alternative Medicine (CAM), Repeated Measures, Sedation, Tetrahydropalmatine (THP).*

## INTRODUCTION

Anesthetics are defined as substances that numb pain or produce a state of sedation (without sensation). The first documented cases of anesthesia date back to the first century where for example the Greek Physician Dioscorides described the use of wine made from the mandrake plant would produce a deep sleep during procedures (Anesthetics 2017). The use of holistic medicine is increasing all around the world, and is used by nearly 20% of the population in the United States (Bent 2008). There are several universities established in the United States that are dedicated to Complementary and Alternative Medicine (CAM) (Association of Accredited Naturopathic Medical Schools). The oldest such school is the "National University of Natural Medicine" located in Portland, OR. It was founded in 1956 by Charles Stone, W. Martin Bleything and Frank Spaulding. According to the *Journal of Health Care for the Poor and Underserved,* a study from 2002 to 2007 showed a significant increase of CAM use. In modern medicine we always look for ways to improve the adverse side effects of, existing (especially synthetic) drugs in use. Because natural medicine is a growing field in the United States it is important to test if compounds extracted from natural materials show less or no side effects compared to some of the medicines that are commonly used.

Some common anesthetics used during surgeries in small animals are Acepromazine, Isoflurane, and Thiopental. Acepromazine is used as a pre-anesthetic to calm down the animal and Isoflurane and Thiopental produce unconsciousness in the animal (Foster/Smith 1997-2016). Side effects of these drugs include chills, vomiting, and in some cases extreme drop in blood pressure and even heart failure (petmd.com 2017). We hypothesize that the negative effects of these drugs may be more severe than the negative effects of dl-tetrahydropalmatine if they exist.

*Corydalis yanhusuo* extract is a mixture of eight alkaloids including corynoline, acetylcorinoline d-corydalin, dl-tetrahydropalmatine, protopine, and tetrahydrocortisine (Ding, et al 2007). Of these alkaloids, research shows that *dl*-tetrahydropalmatine is an alternative to anxiolytic and sedative drugs (Lueng, et al 2003). Of the *d* and *l* configurations, the *l* configuration is said to be the more potent (Bei 2012).

In this study the *d/l* mixture of tetrahydropalmatine was administered to eighteen albino feeder mice in order to test for its sedative qualities. Even though the *l* configuration is more potent, the study used a mixture of the *d/l* isomers as it was more economical. The compound was purchased from Fisher Scientific. Since there is not sufficient research done with this particular compound with mice, caution was taken to determine safe dosages. It is known that too much corydalis can be lethal (Crouse 2013).

## MATERIALS AND METHODS

Twenty-four young Albino feeder mice (2 to 4 weeks old) of random weights purchased from 'Tales and Scales' in Derby KS were used for this experiment. They were kept in cages in the basement of Melhorn hall at room temperature with proper food and water.

Each cage contained cedar bedding and proper lighting was supplied to the mice. All procedures for obtaining the mice, housing them and preoperational and operational protocol were in line with, “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences” (Physiological Society of Japan, 2002). Bedding was changed out as needed. Mouse/Hamster food from Walmart was given to them daily.

The study involved a pilot project prior to the main experiment, in order to determine the suitable range of concentrations of the compound to be given.

The pilot project consisted of two mice, one male and one female. The drug was made into three aqueous solutions of the following concentrations; 22.5g, 45.0g and 67.5g per kilogram of water. Ten grams of each solution was prepared for the pilot study. These concentrations translate into doses of 15, 30 and 45 milligrams of compound per kilogram of mouse. The mice were restrained using one handed restraint method (with rubber gloves) (Restraint and Handling of Animals) and injected with 0.2ml of the 22.5 g/Kg $H_2O$ solution. After three days they were injected with the 45.0 g/Kg $H_2O$ and after three more days with the 67.5 g/Kg $H_2O$ solution. Thirty gauge insulin needles with a capacity of 0.3ml were used for administration. Each injection was followed by a monitoring period which ended when the mice became completely active. The pilot project showed that at the 15 and 30 mg doses, tetrahydropalmatine had no observable effects. It was not until the 45mg dose, that the mice showed signs of sleepiness by resting their heads on the ground and closing their eyes slightly. These observations caused us to raise the experimental concentrations to 75.0g, 92.7g and 110.3g tetrahydropalmatine per kilogram of water which translated to 50, 60 and 70 mg/kg of mouse.

Twenty-four mice were used as test subjects in the main experiment. They were randomly assigned into four groups of six, three experimental and one control. Each experimental group received a certain dose of dl-tetrahydropalmatine. Group 1 received 50 mg/Kg dose, Group 2 received 60 mg/Kg and Group 3 received 70 mg/kg doses. The control group was treated with a neutral solution of HCl and NaOH.

It was determined that three days of rest after each injection would be sufficient for allowing the drug to exit the system. Eight trials were performed to provide enough data for my research. I performed one trial on each group every four days thus after 28 days I had collected all of my data.

**Solution Preparation**

The solutions were made by weighing 37.5, 92.7 and 110.3 mg of *d/l* tetrahydropalmatine for the 50, 60 and 70 mg/kg dose solutions respectively. Sufficient amounts of 0.1 M HCl were added to each solution, making the compound soluble and then 2.0 M NaOH was added to each until they were within two pH points of being neutral. Then double distilled water was added until the weights of the solutions were 5.0, 10.0 and 10.0 g for the 50, 60 and 70 mg/kg dose solutions respectively.

The injection site of each mouse was first disinfected with ethanol and then the drug was administered intraperitoneal in approximately 0.1ml aliquots depending on the weight of the mouse. Weights were recorded before each trial to the one tenth decimal accuracy. During the administration of the drug, single handed restraint was used while wearing rubber gloves. After administration each mouse was monitored for sedation time. Each trial was performed eight times with a three day gap in between trials to allow the compound to cycle out of the body. Each group received the same dose throughout the eight trials in order to minimize variables. Sedation resulting from the tetrahydropalmatine will determine whether the compound exhibits dose dependency and also the sex of each mouse may prove to have an effect on sedation time (Xi-can/Bin 2006).

Sedation was defined by four observed stages the mice underwent based on motor control. Stage 1 – completely active. Stage 2 – calm with some movement. Stage 3 – stationary/resting with head down but responsive to pressure on the head. Stage 4 – resting with head down and unresponsive to pressure on the head. The ‘onset time’ was considered as the time it took from injection to reach Stage 3. ‘Time under’ is the time the mouse spent in Stages 3 and 4. ‘Recovery time’ refers to the time that the mouse spent in Stage 2 while coming out of sedation.

**Analysis**

Repeated measures tests were used to see if there is any statistical significance in the different sedation times between the three treatment groups. Immediately after the data was collected the mice were disposed of according to the following guidelines. The National Institute of Animal Health stresses that lab animals used for research must not be released into the wild but, also if a use cannot be found, humane euthanasia is appropriate disposal of mice. Euthanasia was not needed and the mice were returned to the pet store they were bought from as a donation.

**RESULTS**

This experiment was intended to test the efficacy of *d/l*-tetrahydropalmatine in different doses as a sedative in mice. The different doses delivered intraperitoneal showed to produce results that were dose dependent.

The observation period for each trial was divided into three descriptive times: the onset (time from

injection to stage 3), the time under sedation (stage 3 and 4), and the time for recovery (stage 2 until active). The average time of the eight trials for each of these periods were compared between groups to see if any correlation between dose and time existed.

Average 'onset time' showed a decrease from group 1 – group 3. The average 'time under' increased from group 1 – group 3. The average 'recovery time' decreased from group 1 – group 3. Average Onset times for groups 1, 2, and 3 were 235.8, 145.6, and 140.5 seconds respectively. The average 'Time Under' for Groups 1, 2, and 3 was 1504.5, 2348.9, and 3238.0 seconds respectively. Average Recovery times were 607.6, 559.1, and 502.3 seconds for group 1, 2, and 3 respectively (Table 1).

All of the data was analyzed using the statistical software JASP version 0.8.0.1. Repeated Measures tests were run using only the mice that underwent all eight trials.(Table 2).

Searching for a significant difference between onset times of different doses, the repeated measures test gave a p value of 0.038. The repeated measures for time under gave a p value of < 0.001 and for Recovery time the p value was 0.161. The control group showed no effects from injections throughout all eight trials for all the mice.

**Table 1** Showing Comparison of Averages between three trial groups. Average times for three observational periods of all mice included in the experiment. (Seconds)

| Group | Onset | Time Under | Recovery Time |
|---|---|---|---|
| 1 | 235.81 | 1504.54 | 607.56 |
| 2 | 145.63 | 2348.93 | 559.11 |
| 3 | 140.53 | 3238.00 | 502.31 |

**Table 2** Repeated Measures Statistical Analysis: Comparison of Sedation Times between Groups 1, 2, and 3. Tests for significant differences between Groups. Onset: Time from injection to mouse resting but still responsive. Under: Time spent while mice were stationary and resting. Recovery: time from when mice leave the resting state until they are active.

| Data Set | P value |
|---|---|
| Onset | 0.038 |
| Under | <0.001 |
| Recovery | 0.161 |

## DISCUSSION

Sedation is defined as a drug induced state of calm or sleep. From the average times for the 4 stages of the observation (explained above) we observed that the group treated with the highest concentration of the compound showed effects sooner and stayed in the deepest of the four stages longer than the other groups. The control group did not show any level of sedation which means all effects were result of *d/l*-tetrahydropalmatine.

There were many variables that were involved in this experiment that were hard to account for including the temperament of the individual mouse, the method of injection and the type of mixture (for example – solution vs suspension) that the drug was administered in. Mice that are naturally more "high strung" may have reacted less than others. Intraperitoneal injections do not ensure direct administration and with the size of the mice it was very difficult to say that each injection was delivered exactly in the same manner. Also the drug being in a suspension may have allowed slight variations in concentrations from one injection to the next.

During the course of this experiment, nine mice died. The male in the pilot study and eight males involved in the real experiment all died including the three males in the control group which strongly suggests that the cause of death was not *d/l*-tetrahydropalmatine. Before the eight mice died, I noticed that two mice in one cage appeared to be sick. The next morning, five mice were dead including the two sick ones. The additional three came from adjacent cages. Three more appeared sick and were placed into quarantine where they soon after died. The deaths seemed to be a sort of epidemic which spread from a single source. Only spreading cage by cage, which gave reason to believe that it was an infection. According to an article by Reardon, females tend to have faster immune responses to infections compared to males which could explain why only males died. (Reardon 2016).

Physiologically, *d/l*-tetrahydropalmatine acts as a dopamine $D_2$ receptor antagonist (Chueh, et al 1995). Dopamine; a neurotransmitter involved with motor control and the limbic system, regulates the reward and pleasure centers of the brain. The effects seen in this experiment such as loss of motor control can be attributed to the fact that tetrahydropalmatine inhibits dopamine receptors. There was also a dose dependency observed, meaning the higher concentration of THP (ligand) then the more dopamine inhibition.

The repeated measures tests showed significant difference between the Onset and Time Under of the three doses. Recovery time gave a p value >0.05 which means there wasn't a significant difference between the three doses. When standard Anova tests were conducted comparing the average times the mice spent at each observational stage, including the ones which did not undergo all eight trials, the results showed the same statistical outcome as the repeated measures tests adding support to the findings.

Because the control group showed no effect it was not included in the statistical tests. It was determined that all observed effects were caused by the *d/l*-

tetrahydropalmatine. There was no statistical tests performed to compare results between male and females because the majority of the males died and there was not enough data to analyze.

From the statistical tests, I conclude that *d/l*-tetrahydropalmatine exhibits dose-dependency but in order to observe a deeper level of sedation, a higher dose of dl-tetrahydropalmatine would need to be used. In order to find more information, further studies would need to be conducted and to better account for said variables, a much larger sample size would create a greater experimental power.

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