Evaluate the Influence of Eupatorium adenophorum Extract with Mice Organ

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Abstract: In order to study the influence of extract from Eupatorium adenophorum in mice organs, this experiment will be the basis of further study that make Eupatorium adenophorum become Phyto contraceptive, this experiment take the feeding respectively way after the completion of the 1D, 5D, 10d, 15d of Eupatorium adenophorum mice by intragastrical administration of levonorgestrel group and blank control group. After the same operation in different periods of small rat heart and kidney the uterus, testis, and other organs were observed. The results showed that after extraction of E. adenophorum changes in female mice uterus shape was perfused significantly, showed swelling larger. Data analysis of each viscera coefficient was found E. adenophorum had No obvious effect on the heart, kidneys and testicles of mice. but there are obvious differences date between the treatment group and the blank group. (5d: F=10. 800 P=0. 043 cases) from tissue sections we can see female mice uterus cell morphology changes significantly, there was a similar appearance change in the uterus of the female mice with the estradiol For a male mouse testis of Eupatorium adenophorum gavage had No obvious effect. And it is found that the heart, the treated mice kidney, testis, ovary and other organs were observed in each period of time the organization had No obvious change; only female mice uterus tissue sections of individual cells became larger, and the organization of the gap larger. This research shows that E. adenophorum extract has the potential to develop botanical contraceptives, we will conduct in-depth study.

1. Introduction

The development of agriculture, forestry and animal husbandry are intimidated by plague mice around the word. In recent years, there are 144 million farmers who and 35 million hm2 areas were suffered rat harm each year in China. In addition, the 47 hm2 forest of Northeast China, North china and Northwest China was destroyed. Meanwhile, grassland rodent was increasing. Especially, the mouse can spread many diseases so that it threatened human health all the time. According to statistics, a total of nearly three hundred million people died of plague, it harms far beyond the war. Therefore, how to
control the population of rats has become a common problem in the world. In general, there are three methods to control the mice population: physical, chemical and biological. The physical methods mostly used rattraps or mousetraps, but those equipment are suitable for indoor is Not suitable for large area in the outdoor. It's not only will cause some more damage to the environment, but also do harm to other animals by chemical methods [1-3]. Besides, the chemical can stimulate the rapid propagation of murine, and even recovery the original population number in a short time. At last, the biological methods mainly lead into the natural enemies which has a certain function in a low population density, but it doesn't has much effects when the rats outbreak. Using the above methods have Not achieved remarkable results, so we need change the method and a research direction is murine chemo sterilant. At present, there are some plant rodenticide, such as cabergoline, Tripterygium wilfordii and so on. This study is conducted to observe the effect of E. adenophorum extract on each organ in mice and then evaluating the probability of becoming rodenticides or mice chemo sterilant.

2. Materials and methods.

2.1. Experimental animal
Fifty-day old mice (female 32, male 16) each are healthy. They were purchased from the Chongqing mouse breeding base; the average weight was 50. 5± 4. 7g.

2.2. Experimental reagents
Eupatorium adenophorum extract (ethanol extraction); Levo Norgestrel quinestrol whose manufacturers as Huarun Zizhu Pharmaceutical Co. Ltd and each active ingredient is 9mg; xylene, formaldehyde is used to soak mouse organs. Physiological saline, distilled water; arowana edible oil is used for dissolving Levonorgestrel quinestrol (each with 3 ml edible oil) [9].

2.3. Experimental Equipment
A number of mice cage and sterilized saw blade. Stomach needles were purchased from Internet, and the specifications is No. 16. Many 1ml, 5ml medical syringe. Besides, a set of anatomy tool. Rotary evaporator is used to the extraction of E. adenophorum. The slicer KD-3530 was produced by Cody Technology Co. ltd. Anatomy camera LEICA. Beaker, glass rod, graduated cylinder and so on.

2.4. Experimental methods

2.4.1. Extract production, the stoste of Eupatorium adenophorum was extracted by ethanol solution. At first, make it dry in the shade and chop it. Then, using 500ml alcohol to extract 100g grass clippings, meanwhile, adjust the rotating speed of rotary evaporator to 55r/min, and 60℃. One hour later, filtering, extracting, separating alcohol out. Next, adjusting the rotating speed to 90r/min and 65℃ so that the alcohol evaporates completely. Finally, using the propanetriol to dissolve the remaining, and then set the volume at 100ml.

2.4.2. Gavage time arrangement, 30 female rats and 15male rats divide into three groups which are Eupatorium adenophorum group, Levonorgestrel quinestrol group and blank group, and 10 female mice and 5male mice each group. Continued routine feeding for one week, feeding many times according to arrangement time with 0. 5ml. The time was 1D, 5D, 10D and 15D. What's more, killing two female mice and one male mouse of each group by removal of vertebra in order to anatomical observation and make tissue sections before the 5D, 10D and 15D feeding [4-7]. Especially, ensure adequate daily feed and water during the test. Specific schedule follow Table 1.
Table 1. the time schedule of intragastrical administration and killed

|                      | 1d | 5d | 10d | 15d | 20d |
|----------------------|----|----|-----|-----|-----|
| **Eupatorium adenophora** group | C  | A  | A   | A   | B   |
| **Levonorgestrel quinestrol** group | C  | A  | A   | A   | B   |
| **Blank group**      | C  | A  | A   | A   | B   |

Description:
The A was stand for taking two female rats and one male rat to anatomical observation before gavage, and others was according to the amount to feed.
The B means to remove two female rats and one male rat to anatomical observation
The C was intragastrical administration as usual.

2.4.3. Made and observation tissue sections (1) Fixed: Fixed mouse organs with formaldehyde solution after observation in live and different degrees (cut size is about 3mm×3mm×5mm).
   (2) Dehydration and paraffin embedding: Firstly, soaking 5min as 75%ethaNol, 80%ethaNol, 95%ethaNol (twice) and 100%ethaNol (twice) in sequence. Next, using xylene to soak 15min two times. Finally, taking paraffin embedding.
   (3) Slice: take the embedded various organs to tissue sections and sliced thickness is about 8 micron each.
   (4) Dewaxing: after slicing, the slice was fixed on the slide glass in order to dewax. Specifically, for (2) in the reverse order, and rinse 2min with distilled water at last.
   (5) Hematoxylin stain: after dyeing 5min, rinsing 1min with distilled water. Differentiation 30s in the 1% alcohol and acetic acid, and then washing 5min with distilled water (50 degrees Celsius).
   (6) Eosin stained 15min, and washing with distilled water for a short time.
   (7) Dehydration and mounting: Firstly, sequential immersion in 75% ethanol, 80% ethanol, 95% ethanol (two times), 100% ethanol (two times), each time 5min. Next, soaking 15min in the xylene solution and repeat once. At last, drying 24 hours in the oven at 40 degrees Celsius.
   (8) Observing and taking photos under the microscope.

2.5. Observation index

2.5.1. Observation the morphological variety of each organ. For example, the length, glossiness and hyperemia degree of the uterus; the glossiness of heart and kidney surface, and whether there is a rugged edge.

2.5.2. Determination of the organ coefficient. Weighing the weight of heart, kidney, uterus, testis, and the whole weight of mice by balance. Calculation the organ coefficient. [Organ coefficient (%) = (organ weight / body weight) *100].

2.5.3. Observation the morphology of heart, kidney, uterus and testis. The main objects were the change of tissue space and cell size [8].

3. Result

3.1. The morphology change of each organ

3.1.1. The morphology change of heart, kidney, there is No obvious change with the appearance of female and male rats heart and kidney in E. adenophorum group after repeated feeding. Such as, gloss is Normal, No attachments to the surface of the heart, smooth and No bump.
3.1.2. The uterus morphology change of rats, there is an obvious change with the appearance of female uterus at each period after repeated feeding E. adenophorum which uterine edema. In different periods of E. adenophorum mice uterine morphology diagram as follows (Figures 1 and 2).

![Figure 1. uterus of female rats in different periods (8x)](image)

(The No. 1 was blank control group (no feeding), the No. 2 was 5D (feeding once), the No. 3 was 10D (feeding twice), and the No. 4 was 15D (feeding thrice).

![Figure 2. the uterine changes of female mice in the same period 5D (8x)](image)

Description: it is E. adenophorum group, levonorgestrel and quinestrol group and control group from left to right.

In order to be able, understand, I made the following two tables to reflect the changes in the organs of each male and female mice during different periods (table two) and in the same time difference drugs make different results (table three). There are obvious changes with the number of +, No significant change is used to.

| Table 2. organ changes of male and female mice of the same medicine in different periods |
|---------------------------------------------|
| female | | | | | | | | | | | | | |
| heart | kidney | uterus | ovary | heart | kidney | testis | Spermatic cord |
| 5d | | | | | | | |
| 10d | | | | | | | |
| 15d | | | | | | | |
3.2. Determination of organ coefficient

The experimental data were statistically analysed by Excel 2010, and the data analysis method which named single factor analysis of variance (One-way ANOVA) was used to analyze the conspicuousness of variable difference. When P is less than 0.01, the difference is very significant. When P is less than 0.05, the difference is significant. The mean is mean error ± standard error (Mean ± SE).

3.2.1. Effect of extract of Eupatorium angophora on heart coefficient in mice, the heart coefficient results showed that there was no significant difference among 5D (F2, 9 = 1. 774 P = 0.224), 10D (F2, 9=0.698 P=0.523), 15D (F2, 9=2.311) and blank group.

3.2.2. Effect of extract of Eupatorium angophora on kidney coefficient in mice, the kidney coefficient results showed that there was no significant difference among 5D (F2, 9=0.403 P=0.679), 10D (F2, 9=3.606 P=0.071), 15D (F2, 9=0.322 P=0.733) and blank group.

The Testis coefficient results showed that there was no significant difference among 5D (F2, 3=1.321 P=0.388), 10D (F2, 3=4.971 P=0.112), 15D (F2, 3=2.882 P=0.200) and blank group.

The uterus coefficient results showed that there was significant difference among 5D (F2, 3=10.800 P=0.043), 10D (F2, 3=16.379 P=0.024), 15D (F2, 3=16.555 P=0.024) and blank group.

The Eupatorium angophora Spreng group has No significant difference among 5D (F1, 2=8.1 P=0.104), 10D (F1, 2=5 P=0.155), 15D (F1, 2=7.716 P=0.109 and blank group.

3.3. Histological sections observation

Tissue sections are generally used to study the microstructure of organ and cells, and can be preserved for a long time. It is widely used in medicine, biology, embryology and other fields. According to the contrast, there were no significant changes in the heart and kidney tissue sections of the male and female rats in each group. The renal 10D tissue section of kindey of each group was shown below:

![Figure 3. The renal tissue sections of 10d mice in each group (40x)](image)

Note: The No. 1 is the blank control group 10d; No. 2 for levonorgestrel easters group 10d; No. 3 is E. adenophorum group 10d.
We found that the female rat uterine cells of *E. adenophorum* group and levonorgestrel easters group in different periods have different degrees swelling, taking 10d as a case, after the same magnification observation can obtain the following comparison chart:

![Figure 4. The tissue sections of mice 10d in each group (40x)](image)

Note: The No. 1 is the blank control group 10d; No. 2 for levonorgestrel quinestrol group 10d; No. 3 is Eupatorium adenophora group 10d.

4. Analysis and discussion

4.1. Appearance change observation
After killing mice and observe organs, we found that the heart, kidney and other organs showed no obvious changes in appearance by compared the combination of *E. adenophorum* levonorgestrel group, but in the uterus comparison, we found that the group of levonorgestrel group and *E. adenophorum* group were bigger than the blank group, edema phenomenon. From the reference literature, the effective component of levonorgestrel quinestrol is similar to the EP-1 of (a rodent contraception) which will make the endometrial transformation, thinning, loose interstitial edema, thickening of mucus secretion, being Not conducive to sperm penetration thus causing infertility. From the appearance changes and morphologic changes of *E. adenophorum* group and the group had the similar effect of levonorgestrel quinestrol. The *E. adenophorum* group and the levonorgestrel quinestrol group had the similar effect from the appearance and morphologic changes [10-11].

4.2. Comparative analysis of organ coefficient
From the results of viscera coefficient, there was no significant difference in the heart, kidney and testicular. However, there was a significant difference between each treatment group and the blank group at each time. From the analysis of variance of *E. adenophorum* group and control group, the mean values of P were about 0.05-0.10 so that there is No significant difference. There are some differences of *E. adenophorum* group and control group by the picture analysis. This shows that the extract of *E. adenophorum* spreng has some effects on female mice uterus, but the affect is lower than levonorgestrel and quinestrol.

4.3. Histological observation
From the result of histological observation, it hasn’t obvious changes of the heart, kidney and testis tissue cells from different agent’s mice at the same time in the same group, but uterine tissue sections showed uterine cells occurred swell, and cell gap is also larger. The experimental results demonstrate that the mechanism of levonorgestrel and *E. adenophorum* don’t influence the morphology (size, color and texture) of heart, kidney and testis, but there is a certain effect on the uterus [12-13].

The above three indexes showed that *E. adenophorum* extract had no obvious effect on mouse heart, kidney and testis, but has effect on the uterus which appearance show edema thickening; tissue showed cell edema and big gap. The uterus is the reproductive organs of female mice, so infer the levonorgestrel quinestrol and *E. adenophorum* extract have a certain influence on fertility of female
mice. In a word, this research shows that E. adenophorum extract has the potential to develop botanical contraceptives, we will conduct in-depth study.

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