Effect of Thyme (Thymus vulgaris) on Female Infertility "In Vivo Test on Female Rabbits"

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Abstract - Infertility is a topical problem. Many women have difficulty conceiving; among the main causes is ovarian dysfunction. In most cases, women resort to medicine, but there are those who prefer to use medicinal plants recognized for their fertility power. The objective of our study was to test the medicinal plant "Thymus vulgaris" on fertility in female rabbits. We submitted the rabbits to a "Decapeptyl" to block their ovarian function. Then we tried to unblock it with a stimulant "Purugon" and with thyme. After the sacrifice, we made histological sections to observe the evolution of oogenesis. The results were very encouraging, the microscopic observations of the cuts of the ovaries showed that after the voluntary blockade of oogenesis, the follicular cycle was stopped and there were no mature follicles, then the introduction of the hormone Purugon Activated the ovary again, although the thyme had a similar role as that of the stimulation hormone, for the rabbits who consumed the thyme, there was a restart of the ovogenesis after its blocking. We therefore observed mature eggs ready for ovulation. Therefore, thyme has a fertilizing effect.

Keywords: Female infertility, Thyme (Thymus vulgaris), rabbits, histological section, ovary, ovogenesis, follicles, stimulation.

I. INTRODUCTION

Infertility is considered by the World Health Organization (WHO) as pathology in its own right. Within the concept of reproductive health, that recognizes every human being the right to procreation [12]. There are two types of infertility: primary infertility and secondary infertility. In women, the main etiologies of secondary infertility are infertility due to ovulation disorders, infertility of uterine origin, tubal and pelvic-peritoneal lesions, and endometriosis [4].

The MAP (Medical Assistance to the Procreation) covers all the techniques that medically assist a couple in order to procreate, it has conquered in recent years a place in the panoply of the medical proposals to answer the desire of a child and allowed A considerable advance in the management of infertility [3,14]. The woman is often incriminated, and remains the only one to seek the remedies against the infertility of the couple [1].

In this search for solutions, women most often resort to modern medicine. If she fails, she turns to traditional medicine. While other women use traditional medicine directly for cultural or economic reasons [1, 6, and 10]. It is in this angle that our study has been carried out; we studied a medicinal plant thyme (Thymus vulgaris), very responsive plant in Algeria, to test its therapeutic effect on female infertility.

Our study was carried out as follows: we blocked with a hormone called Decapeptyl in the whole group of rabbits outside the control group, after one week we administered a stimulating hormone called Purugon (subcutaneously) for first group, and for the other Group, we administered thyme orally as an infusion. At the end of the experiment, we sacrificed the animals to extract their genitals, we weighed each animal and these gonads, to calculate the sexual maturity index "condition factor" CF "and the gonadosomatic index "GSI."
We then carried out histological sections of the ovaries in order to make microscopic observations to see how the follicular cycle proceeds to evaluate the functioning of oogenesis and to be able to judge whether after an ovulation problem (such as that caused by the Hormone Decapeptyl), can be restored and restarted a normal ovulation. In order to compare whether the restoration of ovogenesis with the thyme and the same as with the stimulant Purugean. At the end of the study, we assessed the therapeutic reliability of thyme on female fertility.

II. MATERIALS AND METHODS

A. Vegetables materials

Identification of the plant Thymus vulgaris. L was made in the laboratory plant taxonomy of the faculty of sciences of nature and life in the Sidi Bel Abbes, Algeria. Is based the key determination "QUEZEL AND SANTA" (1965)[11, 13].

Animals’ materials

Our study was done on rabbits aged about 2 months and weighing between 2kg and 3kg from the ITELVE institute of Sidi Bel Abbes (Technical Institute of Livestock).

The experiment lasted six weeks (May-June 2016). After a period of adaptation of 06 days, the animals were treated in accordance with the advice on the protection and use of laboratory animals [9]. The humidity of the animal house was 47%, an average temperature of 24-17°C and a photoperiod of 13 h of light and 11 h of darkness, with free access (ad libitum) to water and food (They were fed a standard rodent food that is marketed by the West Poultry Group (GAO ORAVIO-ORAN, Algeria). The workshop was serviced every day.

B. Laboratory equipment

B.1. Inhibition Hormone

We used "Decapeptyl" for males and females. The molecule is the Triptoreline which is an analogue synthetic decapetide natural GnRH (gonadotropin releasing hormone). Studies conducted in humans and in animals have shown that after initial stimulation, prolonged Triptoreline administration leads to inhibition of gonadotropin secretion, thus removing the testicular and ovarian functions.

"Following some animal studies, another mechanism of action was discussed: direct gonadal effect by decreasing the sensitivity of peripheral receptors to GnRH [8].

B.2. Stimulating Hormone

We used Puregon belongs to a group of medicines called "gonadotropins". It contains follicle-stimulating hormone (FSH) in a vial of 50 international units (IU) solution for injection per vial. PUREGON (50 IU / 0.5 ml) is produced from mammalian cells, modified by genetic recombination, so as to carry the genes of the human FSH.

C. Methods Used

C.1. Preparation of plant material

We used three kinds of preparations:

Infusion: which is to put the dried plant and crushed in cold water, then covering fire from the first quivering of the water, they put out the fire, is infused 5 to 10 minutes, then filtered.
Decoction: putting the dried plant and crushed in cold water, which is brought to boiling by covering, 5 to 10 minutes, then filtered.  

Maceration: put the dried plant and crushed in cold water, leave a few hours or days [2, 5]. We first used the powder plants by adopting the 3 above mentioned methods, and for one day, we followed the behavior of rabbits for each preparation, we noticed that the rabbits did not accept the taste solutions prepared, solutions remained intact. We tried the thyme leaves that are not crushed, they are a preparation as an infusion. It was assessed in rabbits; therefore, it was our choice for preparation. We removed the drinking water into three periods: morning, afternoon and evening and we replace it with the infusion of the plant and we quantify the amount drunk with graduate drinkers.  

Preparation of hormonal solutions  

- Decapeptyl is an injectable solution. For an adult dosage is between 75 and 300 units, so we had calculated for a 3kg rabbit, about 2 units, we used syringes with small graduations [7, 14].  
- Purugon is an injectable solution, the same principle as Decapeptyl, we administered 2 units per rabbit because the human dose is about 100 units [7, 14].

C.2. The distribution of prizes  

The distribution of different batches is based on the purpose of blocking ovarian functions of female rabbits, then proceeded to release one hand with synthetic hormones and secondly with the tested plants to make a comparison operation of the genitals, therefore, to judge the effect of Thyme on female fertility. Four (4) prizes for females (12 rabbits)  
Lot 1: Witness rabbits (3 rabbits)  
Lot 2: Inhibition (Decapeptyl) + stimulation (Purugon) (3 rabbits)  
Lot 3: Inhibition (Decapeptyl) without stimulation (3 rabbit)  
Lot 4: Inhibition (Decapeptyl) + stimulation with thyme (3 rabbits)  
Preparations plants were administered in drinking water.

C.3. Removal of the ovaries and testes  

After the sacrificing of the animals, the ovaries were gently removed and weighed and then placed in 125 ml labeled conical polypropylene jars filled with 35% formalin.  

C.3.1. Ovarian evaluation  

The evolution of the weight of the ovaries was evaluated on the basis of the calculation of the sexual maturity indices of the rabbits experimented with the controls.
The index of sexual maturity

The maturity indices are parameters related to the physiology of the animal, and can give us indications on the state of the reproductive system, or sexual behavior. In these studies we used two indices; the condition factor “CF” and Gonadosomatic index “GSI” according to the following two equations (breeding test protocol Environment Canada, 2010) [9]:

\[
CF = \frac{\text{Animal Weight (g)}}{\text{Animal length (cm)/10}^3} \times 100 \quad [9].
\]

\[
\text{GSI} \% = \frac{\text{Gonad Weight (g)}}{\text{Animal Weight (g)}} \times 100 \quad [9].
\]

C.3.2. The stages of the histological study

Ovaries removed our guinea pigs will undergo several stages to finish in a blade fixed in order to observe the course of genesis observing different stages of follicles in the ovary.

There are five steps to achieve a histological section:

a. Fixing

This first stage starts immediately after collecting the member (ovary), its role is the conservation of different tissue of the organ, it prevents cell autolysis, also prevents bacterial putrefaction and allows histological technique and colorings later. We used formalin 35%.

b. The inclusion

The principle of inclusion is to have a rigid consistency tissue, in order to achieve very fine cuts to allow the passage of light through the fabric, in order to have a good microscopic observation. The paraffin embedding includes the infiltration and coating the tissues to be examined with paraffin. We proceed before this coating of two mandatory stages. The first step is dehydration where we will spend the tissues in growing degree of alcohol baths (70 °, 80 °, 90 °, 95 °, 99 °, and finally 100 °), the interest of dehydration is to remove the fixative. In the second step, we proceed to toluene bath which is a solvent miscible with paraffin to replace the alcohol. Finally the fabric is placed in the molten paraffin, heat will accelerate the evaporation of the solvent, then placed in small molds, at room temperature, it will harden, we turn out to obtain fractions of paraffin tissue.

Using a microtome cuts is performed. The instrument moves the block on a knife. The cuts are about 5 microns. All tranches will form a tape in which we find the tissue sample cups. One makes a spreading section on glass slides, heated on a hot plate the paraffin will stick to the blade.

c. The colorations

The most commonly used stain is hematoxylin / eosin / saffron (HES). Haematein is a basic substance, which stains nuclei purple therefore stained nucleic acids. Eosin is an acidic substance, which stains pink cytoplasm, it is the protein staining. Saffron color the yellow collagen fibers. Prior to staining, there is a dewaxing, one passes the blades in toluene baths. Then this is rehydration: the alcohol is mixed with water and toluene, we pass the blades in alcohol baths of decreasing degree (100 ° to 70 °).

d. The mounting

The slides were dehydrated through baths in toluene and then glue glass coverslips over with synthetic resins to preserve the preparations. These slides can be stored for several years [7].
III. RESULTS AND DISCUSSION

In our study we evaluated the effect of thyme on rabbits (females). According to the experimental protocol, we divided our animals into 4 batches: the first one was the control and the other three batches were subjected to an inhibitor of the ovogenesis "Decapeptyl" in order to block the gonadotropic secretion, thus suppressing the functions of the ovary. After one week, one of these three batch undergoes an unblocking by stimulation with the pharmaceutical molecule "Purugon", the other batch is that which consumed the Thyme and the third batch remained without stimulation. Then the results revealed the following findings:

A. Effects of thyme on the weight evolution of rabbits

On the first day of receiving the rabbits we took their initial weight. For a period of one month we experimented on them infusions of Thyme (Thymus vulgaris). Before the sacrifice we weighed them again to have their final weight. The results obtained are shown in FIG.1

We thus found that the largest weight gain of 670 g was observed on the rabbits who were subjected to a blockage with "Decapeptyl" then to a stimulation with "Purugon", followed by the rabbits of the batch which suffered only an inhibition with "Decapeptyl" with a weight gain of 330 g and last it is the control batch and the one where the rabbits consumed Thyme with weight gains of 298 g and 260 g respectively. These results lead us to say that thyme does not really affect the weight growth of rabbits.

B. Condition factor "CF" of the different batches of experimentation

After the one-month experimentation, we tested the effect of Thyme on the reproduction of the rabbits, we made the sacrifices of the rabbits, measurements of the weights and the lengths were carried out for each rabbit. We computed the CF condition factors using the following equation:

\[
CF = \frac{\text{Rabbit weight (g)}}{\text{Rabbit length (cm)} / 10^3} \times 100 \quad (\text{Environnement Canada, 2010}) \ [9].
\]

FIG. 1: Weight evolution of the experimented rabbits.
We found that there is not a really significant difference between the different lots. Between the control batch and the one where we gave Thyme, the CF is almost identical. Hence we can say that there is no effect of our plant on the condition factor that binds the weight of the animal with its size. Figure 2.

![EFFECT OF THYME ON THE CF RATIO IN FEMALE RABBITS](image)

**FIG. 2**: Condition factor in rabbit’s ♀ after consumption of Thyme.

C. Gonadosomatic Index “GSI” of the different batches of experimentation

After the sacrifice of the rabbits, in addition to measurements of the lengths and weights of the rabbits, we also weighed the gonads, so we calculated the GSI according to the following formula:

\[
\text{GSI} \% = \frac{\text{Gonad weight (g)}}{\text{Rabbit weight (g)}} \times 100 \text{ (Environnement Canada, 2010)} \quad [9].
\]

We found that GSIs do not show a remarkable difference between the different batches. However, we observed a small increase for the lot where the rabbits consumed thyme infusions, where GSI is 0.0160% (see Figure 3). Although this is only a small difference, it does not stop us from saying that Thyme has an effect on sexual maturity.

![EFFECT OF THYME ON THE GSI REPORT IN FEMALE RABBITS](image)

**FIG. 3**: Gonadosomatic index in rabbit’s ♀ after consumption of Thyme.
D. Effects of Thyme on ovarian histology of rabbits

After the experimental period our rabbits of the various batches were sacrificed, in addition to the weights and lengths of the bodies and gonads of the rabbits, we also performed histological sections of the ovaries of the experimental rabbits.

The end of our study was the microscopic observation of the fixed plates prepared.

The microscopic study illustrated the state of ovogenesis in the rabbits of the different batches. Starting with the control case (see FIG 4). We noticed the turn of the ovary containing a large number of follicles at different stages of maturity. We also noticed that there are several antral follicles, inside the follicle, we saw very clearly the oocyte and its nucleus. Concerning lot 2 which has been blocked with a decapetide (see FIG. 5), we have noticed that there is no antral follicle, so no ovogenesis is evident. As a result batch 3 where we administered "Purugon" after blocking, which is a stimulant (see FIG 6), we noticed a triggering of the ovarian cycle, we again distinguished normal forms from the antral follicles. And finally, concerning batch 4 which is the most important for our study where we administered thyme (see FIG.7), then the results show anovogenesis which takes place in a normal way with follicles at different stages of their development and Several mature follicles waiting for ovulation in a rabbit is after the protrusion. From this result, we can say that thyme has a stimulating effect inovogenesis in rabbits.

![FIG. 4 : Histological section of the ovaries of the rabbits of the control group seen under the optical microscope
A: (G = 40) B: (G = 100) C: (G = 400)](image-url)
FIG. 5: Histological section of the ovaries of rabbits in the batch subjected to inhibition with "Decapeptyl"
D: (G = 40) E: (G = 100) F: (G = 400)

FIG. 6: Histological section of the ovaries of rabbits in the batch subjected to stimulation with "Purugon" after blocking
G: (G = 40) H: (G = 100)
Infertility is an absence of conception after a year of regular sexual intercourse; it is considered a public health problem, although it is not considered a disease. Several environmental and behavioral factors have unfortunately caused an increase in the phenomenon of infertility in couples. Several studies have shown that there is a significant increase in fertility problems worldwide over the past few years. Although science is in continuous progress, and there have been remedies to overcome the obstacle of conception, but that did not solve the problem, on the one hand the chances are minimal, and on the other hand these solutions are very expensive. Our study was aimed at assessing the effect of traditional medicine plants on fertility, we chose Thyme (Thymus vulgaris) as a plant, and we used rabbits (female) as laboratory animals.

Our experimentation took place as follows: After one week of adaptation, rabbits were divided into 4 batches, the control and three other batches which were blocked by ovogenesis for one week by a "Decapeptyl" inhibitor. The 3rd and 4th weeks, two batches were subjected to a stimulation, one batch by a stimulator "Purugon" the other batch by the infusion of Thyme, while the last batch it remained without stimulation. After the sacrifice of the rabbits, we took weights and lengths in addition to the weight of the ovaries to calculate the indices of sexual maturity.
The last phase was the realization of the histological sections of the gonads of experimental rabbits. The results obtained allowed us to draw the following conclusions:

- As regards the weight growth of the rabbits during the experiment, the results obtained showed that the most important weight gain is that of the batch which has undergone a stimulation with a gain of 670 g, whereas the one who has consumed the infusion of thyme showed the lowest weight growth (260 g), according to which thyme had no effect on the weight growth of rabbits.

- As for the sexual maturity indices, we obtained CFs very similar in the different batch a small elevation for the lot that has undergone inhibition, for the GSI we have noticed that the highest percentage is that of the batch which has received Thyme. This explains why it has a positive effect on the maturity of rabbits.

- Observing the histological sections of the ovaries, germinal cells have been observed at different stages of development and therefore reactivation of ovogenesis after decapetyl-induced blockade, so the effect of thyme was as important as the pharmaceutical stimulant "Purugon".

These results show that thyme has a stimulating effect on ovogenesis in rabbits. Similar studies by Miloš Nikolić [16] have shown that essential oils of the thymus have an effect on cystitis and urethritis, and as we know that one of the most common causes of infertility in a woman are cystitis. So these results agree with us, and we can say that thyme works against female infertility [15,16,17].

REFERENCES

11. Hadef Y. Composition chimique et activité antifongique des huiles essentielles de thymus vulgaris L. et thymus numidicuspoiretd’algérie : l’ère de la pharmacie sur les plantes médicinales;Annaba 08-09-2004.