

Effects of aqueous extract of curled leaves of *Petroselinum crispum* (Mill.) Fuss (Apiaceae) 1925 on reproductive performance of female rats

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Abstract

The present study is part of a program to valorize the medical flora in order to help the populations to benefit from the use of food and medicinal plants. *Petroselinum crispum* (Mill.) Fuss (Apiaceae) is a plant with biological properties. This work was carried out to evaluate the effect of aqueous extract of curly leaves of *Petroselinum crispum* on the reproductive performance of rats. To carry out this study, 32 female rats were divided into four batches of eight (8) rats (8 rats/batch). Each batch was subdivided into two batches of four animals (4 rats/batch) and treated orally at two and four weeks, respectively, and then put into reproduction after the treatments. Lot 1 (control) of each received distilled water and lots 2,3 and 4 of each lot received 50, 100 and 1000 mg/kg body weight of *Petroselinum crispum* extract, respectively. The results revealed 100 % improvement in fertility and 625 and 700 % improvement in fecundity of the rats treated at 50 and 100 mg/kg body weight respectively. At the dose of 1000 mg/kg body weight, the fertility and fecundity index was 0 %. The aqueous extract of *Petroselinum crispum* has no negative effect on the growth of pups born to female rats treated at 50 and 100 mg/kg body weight. After four weeks of treatment, the fertility rate and fecundity decreased.

Key words: *Petroselinum crispum*, performance, reproduction, fertility, fecundity, female rats

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I. Introduction

The world's population is facing problems with reproduction, which is the faculty by which living things perpetuate themselves. These problems are due to infertility, which has become a public health problem¹. Indeed, about 45.8 million couples worldwide have difficulties in having a child after five years of marital life². In sub-Saharan Africa, 30 % of women suffer from infertility. In Ivory Coast, infertility affects about 14.03 % of couples. Infertility deserves to be managed sensitively because it is often a source of instability, polygamy or divorce in some households³. In view of the low income of rural populations, medicinal plants are traditionally used as an alternative solution in the treatment of fertility in several regions of the world⁴. Indeed, the plant world is an excellent source of active principles such as alkaloids, flavonoids, heterosides, which gives it important biological activities often sought in alternative medicine⁵. In addition, the Apiaceae family, formerly called Umbelliferae is known for its richness in essential oil and these multiple metabolic compounds⁶. In order to determine the role of this plant in animal reproduction, the objective of this study is to verify the impact of *Petroselinum crispum* on reproductive performance in small mammals whose experimental model is the female rat *Rattus norvegicus*.

II. Material and Methodology

Plant material

The plant material used was fresh curled leaves of *Petroselinum crispum* (Mill) Fuss (Apiaceae). The leaves were collected in the south of Abidjan (Ivory Coast). A sample of this plant was authenticated by the Herbarium Manager of the National Floristic Center (CNF) of Cocody of the University Félix Houphouët Boigny of Cocody-Abidjan under the number STR14453.

Animal material

The animals used are adult female rats of the species *Rattus norvegicus* (Muridae) of Wistar strain. They are two to three months old and nulliparous. The rats were caged and acclimated in the Vivarium of the School Normal Superior (SNS) in Abidjan. The ambient temperature was 26-30°C, humidity was 40-60% and lighting was 12 hours of darkness. Rats were fed ad libitum. The food consisted of FACI® pellets, corn, bakery bread and dry fish.

Methodology

The reproductive performance of female rats was assessed by fertility rate, fecundity, prolificity, numerical productivity. Thirty-two female rats were divided into four batches of eight (8) rats (8 rats/batch). Each lot was subdivided into two lots of four animals (4 rats/lot).

- First batch; each animal received distilled water at 1ml/100g of mc (Batch 1 control).
- Second batch; each batch of animal was treated with the daily dose of 50 mg/kg body weight of the aqueous extract of *Petroselinum crispum* (Batch 2).
- Batch 3; each batch of animals was treated with 100 mg/kg body weight daily of the aqueous extract of *Petroselinum crispum* (Batch 3).
- Batch 4; each animal was treated with a daily dose of 1000 mg/kg body weight of the aqueous extract of *Petroselinum crispum* (Batch 4).

The batches were treated daily for two and four weeks respectively and put into breeding.

Mating

After two and four weeks of daily treatment, the animals of the different batches were mated. In fact, two experienced adult males were introduced for 10 days for mating in each batch; separating them in the ratio of two female rats for one male rat. After the 10 days with the male, the females were separated from the males and placed individually in cages. They were fed and watered ad libitum until their expected whelping date.

Pregnancy detection

Every morning at the same time, vaginal smears were performed on each female rat after the introduction of the male rat. Thus, a 9‰ (v/v) saline solution of approximately 10 µL was carefully introduced into the vagina with a micropipette and then collected and observed under a light microscope⁷. The presence of sperm in the vaginal stream of a female rat is synonymous with pregnancy and this day is considered day 1 of gestation. This female rat is weighed and removed from the mating cage.

Fertility rate

Fertility is the ability of the female to be fertilized during estrus. The inability of this function is referred to as transient or permanent infertility including sterility. On this scale, the calculation of the fertility rate is as follows:

$$\text{Fertility rate} = \frac{\text{Number of gestating females}}{\text{Number of females reproduced}} \times 100$$

Fecundity rate

Fecundity is the ability of a female to give a live birth. It can also be measured by the number of live animals born to a female during her breeding career. For this study, the fecundity rate is determined as follows

$$\text{Fecundity rate} = \frac{\text{Number of kids alive}}{\text{Number of females reproduced}} \times 100$$

Prolificity rate

Prolificity is the ability of a female to give birth to one or more live offspring during one farrowing. According to this formula, the prolificity rate is

$$\text{Prolificity rate} = \frac{\text{Number of live births}}{\text{Number of births}} \times 100$$

Numerical productivity rate

The numerical productivity rate is closely related to the growth mortality rate and the stillbirth rate. The effect of the aqueous extract of the leaves of *Petroselinum crispum* cannot be satisfied with having a high fecundity rate without having a maximum number of young born. Therefore, in order to take into account the incidents that may occur after birth, we can retain what is called the numerical productivity rate⁸.

$$\text{Numerical productivity rate} = \frac{\text{Number of young weaned}}{\text{Number of females reproduced}} \times 100$$

Statistical analysis

Statistical analyses of the experimental results were performed using GraphPad Prism 7.1 software (Microsoft, USA). Values are presented as mean \pm standard error. Data were evaluated by the one-factor ANOVA analysis method followed by Tukey's multiple comparison test at the 5 % level to assess the significance of observed differences.

III. Results

Effect of Petroselinum crispum on fertility rate

The administration of the aqueous extract of the leaves of *Petroselinum crispum* with the doses of 50 and 100 mg/kg body weight after two weeks showed a very high fertility rate of 100 % or 4 pregnant rats for every 4 mated rats against 50 % or 2 pregnant rats for every 4 mated rats in the controls. This highly significant percentage ($p < 0.01$) shows that all rats were pregnant before the 10th day after treatment with the respective doses.

In rats treated with 1000 mg/kg body weight the fertility rate index was 0 %, which means that none of the female rats were pregnant after two weeks of treatment with aqueous extract of *P. crispum* compared to the control group with a rate of 50 % or 2 pregnant rats for every 4 rats bred (Table I). In addition, after four weeks of treatment, the 50 and 100 mg/kg body weight dose levels showed a 50 % reduction in fertility or 2 pregnant rats per 4 mated rats and a 75 % reduction or 3 pregnant rats per 4 mated rats, respectively. In animals treated with 1000 mg/kg body weight, a zero fertility rate with an index of 0 % was always observed compared to control female rats with a rate of 50 % (Table II).

Effect of Petroselinum crispum on fecundity rate

The fecundity rate after two weeks of treatment with the aqueous extract of *Petroselinum crispum* leaves at the doses 50 and 100 mg/kg body weight increased very significantly ($p < 0.01$) with a respective fecundity index of 625 and 700 % compared to 275 % in the control group. However, the treatment of female rats at 1000 mg/kg body weight showed no fecundity rate with an index of 0 %, this means that no animal midwived after two weeks of treatment with aqueous extract of *P. crispum* (Table I). After four weeks of treatment with aqueous extract of *P. crispum* leaves the doses of 50 and 100 mg/kg body weight showed 300 and 500 % fertility compared to the control respectively. The dose of 1000 mg/kg body weight gave a significantly lower ($p < 0.05$) zero fecundity rate or index of 0 % compared to the control animals (Table II).

Effect of Petroselinum crispum on prolificacy and numerical productivity

To remove the last equivocation of the aqueous extract of the leaves of *Petroselinum crispum*, the prolificacy rate and numerical productivity, also evolved after two weeks and four weeks of treatments. The treatment of female rats at 50 and 100 mg/kg body weight showed a highly significant ($p < 0.01$) prolificacy rate of 625 % or 25 pups per 4 pregnant rats and 750 % or 30 pups per 4 pregnant rats, respectively, after two weeks of treatment compared to the control with a rate of 550 % or 11 pups per 2 pregnant rats. However, the 1000 mg/kg body weight dose showed significantly lower prolificacy and productivity ($p < 0.05$) with a 0 % index each.

Treatment of female rats with *P. crispum* curly leaf extract at 50 and 100 mg/kg body weight showed a numerical productivity rate of 600% or 25 pups per 4 pregnant females and 725 % or 30 pups per 4 pregnant females respectively. The rate of numerical productivity increased very significantly ($p < 0.01$) at 50 and 100 mg/kg body weight, respectively, compared to 250 % in female rats in the control group and 00 % in rats treated with 1000 mg/kg body weight (Table I). After four weeks of treatment of rats, the 1000 mg/kg body weight dose gave no prolificacy and numerical productivity with for both parameters compared to the control. Treatment with aqueous extract of *P. crispum* leaves at 50 and 100 mg/kg body weight resulted in a prolificacy rate of 700 % and 666.67 % respectively. The treatment of rats with *P. crispum* leaf extract at doses of 50 and 100 mg/kg of body weight resulted in an increase in numerical productivity rate of 600 % and 500 % respectively compared to the control group which is 300 %. This increase in numerical productivity rate was highly significant ($p < 0.01$) at 50 and 100 mg/kg body weight respectively compared to 300 % in control female rats (Table II).

Table I: Effects of *Petroselinum crispum* on the reproductive performance of female rats after two weeks of treatment

Treatment at EAPC (mg/kg de bw)	Control	Dose 50	Dose 100	Dose1000
Number of pregnant rats	2/4	4/4	4/4	0/4
Nombre Number of ratons born	11	25	30	00
Fertility rate (%)	50	100	100	00
Fecundity rate (%)	275	625	750	00
Prolificity rate (%)	550	625	750	00
Numerical productivity rate (%)	250	600	725	00

The female rats were given different doses of aqueous extract of *Petroselinum crispum* (EAPC) for two weeks. Values are means \pm MSE (n=4/lots); bw: body mass

Table II: Effects of *Petroselinum crispum* on the reproductive performance of female rats after four weeks of treatment

Treatment at EAPC (mg/kg de bw)	Control	Dose 50	Dose 100	Dose1000
Number of pregnant rats	2/4	2/4	3/4	0/4
Number of ratons born	12	14	20	00
Fertility rate (%)	50	50	75	00
Fecundity rate (%)	300	300	500	00
Prolificity rate (%)	600	700	666.67	00
Numerical productivity rate (%)	300	600	500	00

Control of Female rats were given different doses of aqueous extract of *Petroselinum crispum* (EAPC) for four weeks. Values are means \pm MSE (n=4/batch); bw: body mass

IV. Discussion

Administration of the aqueous extract of *Petroselinum crispum* leaves with the doses 50 and 100 mg/kg body weight to rats showed 100 % fertility and fecundity rate. The high fertility index and fecundity index in the present study would indicate, the estrogenic and progesteronic effects of the aqueous extract *Petroselinum crispum*. Indeed, estrogen activates estrus, contributes to the stimulation of ovulation and is responsible for the proliferation of the uterine epithelium⁹. Progesterone, on the other hand, promotes the proliferation of uterine glands that are essential for implantation and subsequent maintenance of pregnancy¹⁰. The variation in results between the different batches would be due to the dose-dependent effect of the aqueous extract of *Petroselinum crispum*. These data are in line with the effects induced by the aqueous extract of *Moringa oleifera* on the estrous cycle of female rats¹¹. The increase in the number of live-born pups would confirm the presence of multiple fetal implantation sites¹².

Female rats treated for two and four weeks and placed in the presence of untreated males, showed a significant increase in body mass on day 15 of gestation compared to the control group. This increase in body mass is believed to be due to the gestational status and number of fetuses carried by female rats. These data are in line with those observed by Zougrou et al.¹³. Indeed, these authors observed a significant increase in the body mass of pregnant female rats, evaluating the fertilizing and embryotoxic effects of the aqueous extract of *Cnestis ferruginea* leaves in female rats at doses 50 and 100 mg/Kg body mass. The results of the copulation delay showed that before the 10th day of mating, all the animals treated at the doses 50 and 100 mg/kg body mass were pregnant with a fertility index of 100 %. Indeed, the aqueous extract of *P. crispum* leaves maintained the estrous cycle of the treated female rats in estrus, making them permanently receptive. These results are similar to those obtained by Kouakou et al.¹¹.

Fecundity, prolificacy and numerical productivity were elevated in the 50 and 100 mg/kg body weight treated lots compared to the control lot. These results are similar to those observed in the experimental study of the effect of whole root infusion of *Nauclea latifolia* on reproductive performance in treated female rats¹⁴. On the other hand, these results obtained are superior to those recorded by Kenmogne on Wistar strain rats⁸. This difference observed between our results and those obtained by Kenmogne can be explained by the fact that at the time of breeding, the animals used in this study were still young. Indeed, some authors have shown that in most mammals, a decrease in fecundity is observed with age¹⁵. This age is mainly the result of the aging of the uterine preimplantation response. In rats, ovarian activity progressively decreases and stops well before the end of life. Also, the body condition factor (weight loss or excess weight) is one of the most important determinants of reproductive performance¹⁶. In this study, the values of the reproductive parameters observed in treated female rats were quite high, and approximately the same between the 50 and 100 mg/kg body weight doses compared to those of control females. The differences observed between the reproductive parameters with the two doses were statistically insignificant. Thus, the plant did not induce follicular atresia or sterility at 50 and 100 mg/kg body weight. In other words, the androgenic substances contained in the aqueous extract of *P.*

crispum leaves did not reach the level of concentration that could affect the germinal function of the ovary. The improvement of reproductive performance in female rats treated with aqueous extract of *P. crispum* leaves and mated to males could be due to the estrogenic and antioxidant potentialities of this plant. These results also corroborate those obtained by other authors with the aqueous extract of *Cnestis ferruginea*¹³.

On the other hand, the fertility and fecundity indexes on reproductive performance with the dose of 1000 mg/Kg of body mass is 0 %. This result could be explained by the fact that the aqueous extract of *P. crispum* would have androgenic effects at high dose. Or it could be related to a too high estrogen level. Indeed, biologically, androgens act at the level of the ovary to oppose the trophic effect of estrogens on follicular maturation and proliferation of granulosa cells, thus leading to follicular atresia¹⁷.

The variance results show that at birth, the mean number of pups born to females treated with 50 and 100 mg/kg body weight mated to males is significantly ($p < 0.05$) higher than that of pups born to control females. These results are contrary to those obtained with *Allium sativum* aqueous extract, which induced no significant difference ($p > 0.05$) between the number of pups born to the treated females and those of the control group¹⁸. For both doses, the pups born to the females of the treated female rats in our trials have conforming masses.

V. Conclusion

Aqueous extract of *Petroselinum crispum* leaves improves fertility and fecundity by increasing the number of pregnant rats, the number of implantation sites and the number of viable fetuses in treated female rats. The extract of this plant induces an increase in the fertility and fecundity index with the doses of 50 and 100 mg/kg of body mass. However, the effect of this plant gives opposite results at the dose of 1000 mg/kg of body mass. The latter has therefore a total irreversible effect. The aqueous extract of the leaves of *Petroselinum crispum* does not act negatively on the growth of pups born to females treated with the doses of 50 and 100 mg/kg of body mass. After four weeks, the fertility rate and fecundity decreased. The results obtained show that the administration of the aqueous extract of the leaves of *Petroselinum crispum* to female rats allows to improve the reproductive performances of the female rats.

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