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Phytochemical screening and evaluation of the estrogenic effect of the aqueous extract of the curled leaves of *Petroselinum crispum* (Mill.) Fuss (Apiaceae) 1925 on the reproductive system of female rats

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Abstract

The present study is part of a vast program of valorization of the medicinal flora in order to help the populations to draw a real profit from the use of the plants. The objective of this work was to determine through a phytochemical screening, the main chemical groups and the evaluation of the estrogenic effect of the aqueous extract of the curled leaves of *P. crispum* in rats. To achieve this objective, a tri-phytochemistry was performed on the aqueous extract of *P. crispum* leaves and compounds such as phenols, flavonoids, catechic tannins, saponosides, sterols and polyterpenes were investigated. For the study of estrogenic properties, four groups of six rats were formed and treated orally. Group 1 received distilled water and constituted the control lot. Groups 2, 3 and 4 received the aqueous extract of *P. crispum* leaves at doses of 50, 100 and 200 mg/kg body weight, respectively. The results of this study revealed that the aqueous extract of *P. crispum* leaves maintained the estrous cycle in estrus and significantly increased ovarian mass and uterine horn. The concentration of reproductive hormones increases significantly at doses of 50 and 100 mg/kg body weight. At these doses, the extract also stimulates follicular development and ovulation, which are at the origin of the estrus phenomenon. Thus its traditional use in the treatment of infertility is justified.

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Introduction

The use of medicinal plants and herbal preparations has been increasingly successful in recent years (Dibong *et al.*, 2011). The success of these medicinal plants in the treatment of several pathologies has led the pharmaceutical industries to become increasingly interested in the ethnobotanical study of plants. The synergy of action between traditional medicine and pharmaceutical companies is now bearing fruit. Indeed, about 30% of the drugs prescribed by medicine are of natural origin. Better still, this proportion is 50% for over-the-counter drugs (Anthoula, 2003). The curled leaves of *Petroselinum crispum* consumed in Europe since the Middle Ages are increasingly used in traditional medicine (Yanardag *et al.,* 2003; Behtash *et al.*, 2008). Today, *P. crispum* is sold commercially in Ivory Coast in fresh and dried forms (Rayment, 2016). Due to its use in traditional medicine for the treatment of certain infertility-related pathologies such as dysmenorrhea, functional amenorrhea and management of menstrual disorders, this study will evaluate the estrogenic effect of the aqueous extract of curly-leaf *P. crispum* on the reproductive system of female rats.

Materials

Plant material

The plant material used was fresh curled leaves of *Petroselinum crispum* (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 Training and Research Unit (UFR) Biosciences of the University Félix Houphouët Boigny of Cocody-Abidjan under the number STR14453.

Animal material

The animals used were female rats of the species *Rattus norvegicus* (Muridae) of the Wistar strain. They were three to four months old, nulliparous and non-pregnant, with a body mass between 120 and 140 grams. These animals came from the Vivarium of the School Normal Superior (SNS) of Côte d'Ivoire. The ambient temperature was 26-30°C, the 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

Aqueous extract of Petroselinum crispum

The fresh curly leaves of *Petroselinum crispum* were harvested in August and dried for three weeks in the sun at room temperature (30 ± 2°C) and then ground with an electric grinder type IAMAG-RCT® to obtain a powder. The powder obtained is macerated by mixing 50 g in 1.5 liters of distilled water. The whole is agitated three times for three minutes in a Single brand blender (Singapore). The obtained macerate is filtered four times on poplin cloth and then four times on absorbent cotton (Zirihi *et al.*, 2003). The filtrate was evaporated in an oven at 50°C for 48 hours. A dry extract of *Petroselinum crispum* (EAPC) was obtained and used to perform the different tests.

Phytochemical screening of Petroselinum crispum.

Phytochemical screening is an analysis based on standard staining and/or precipitation reactions. The analytical technique described in work is the one used by some authors (Békro *et al.*, 2007)

Effects of Petroselinum crispum extract on the reproductive system

Technical of selection of female rats

This part of the experiment lasted 10 days and is intended to select female rats with a regular cycle for further experiments. Every morning at 7:00 am, vaginal swabs were taken from a total number of 40 virgin rats. The vaginal smears were performed to monitor the estrous cycle of the female rats.

The high level of basophilic and eosinophilic cells in the different smears indicate proestrus and estrus, respectively. The maximum of leukocytes determined indicates metestrus or diestrus. At the end of the 10 days, female rats having presented two successive cycles of four or five days duration alternating estrous phases in the order of proestrus, estrus, metoestrus and diestrus are selected. They are considered to have a regular cycle.

Technical of vaginal smear

Sampling is done using a cotton swab moistened with 9 NaCl physiological fluid ‰. The cotton swab is gently inserted into the vagina of the female rat without stressing it and then rotated in the same direction until there is slight resistance (Sahar and Abeer, 2007). The sample is immediately spread on a clean microscope slide. A drop of methylene blue (2%) diluted 1:10 is placed on the slide on which the vaginal swab has been spread. The preparation is covered with a coverslip; then the examination is done two (2) minutes after the deposit of the drop so that the sample is impregnated by the dye. The different cell types are eosinophils, leukocytes and basophils. The slides were examined under a light microscope by counting 200 cells on the slide (Kouakou *et al.,* 2018). Thus, the percentage of three cell types based on the staining of the nucleus and its shape is determined. By calculating the percentages of the different cell types, the different phases of the estrous cycle can be determined.

Treatment of animals

The administration of the aqueous extract of the leaves of *P. crispum* was carried out on 24 female rats with a regular cycle. This experiment lasted 30 days. The pre-selected female rats were divided into four batches of six animals (6 female rats/batch), including one control batch and three treated batches. In lot 1, which is the control lot, the animals received only distilled water. Batches 2, 3 and 4 received 50, 100 and 200 mg/Kg of body weight of the aqueous extract of *P. crispum* leaves, respectively. In each batch, the animals received 1 ml/ 100 g body weight of the aqueous extract of *P. crispum* leaves orally.

Determination of hormones

Using an LC-04B PLUS centrifuge (China), blood from female rats collected in tubes without anticoagulant was centrifuged at 4000 rpm for 10 minutes. The serum was collected in Eppendorf tubes for pituitary and gonadal hormone determination. The FINECARE FS-112 (China) was used for the determination of the reproductive hormones FSH, LH, estrogen and progesterone.

Statistical analysis

Statistical analyses of the experimental results were performed using Graph Pad Prism 7.1 software (Microsoft, USA). Values were presented as mean ± standard error on the mean. 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 the observed differences. Graphical representations were made using the same software.

Results

Phytochemical screening of the aqueous extract of Petroselinum crispum

Phytochemical analysis of the aqueous extract of *P. crispum* leaves revealed the presence of phenols, flavonoids, catechic tannins, saponosides, sterols and polyterpenes, alkaloids and quinone substances. In contrast, gall tannins were not found in the aqueous extract of *P. crispum* (Table 1).

Table 1. Chemical composition of *Petroselinum crispum* leaves.

Stérol/ Polyterpène	Phenol	Flavonoid	Tannins		Quinone substance	Alkaloid	
			Catechetical	Galic		Dragendorff	Bouchardat
+	+	+	+	-	+	+	+

+: Presence of phytochemicals; - : Absence of phytochemicals.

Effects of aqueous extract of P. crispum on the estrous cycle of female rats

The administration of the aqueous extract of the leaves of *Petroselinum crispum* at doses of 50, 100 and 200 mg/Kg of body weight, caused a disruption of the estrous cycle of female rats. This disruption was marked by a significant increase ($p < 0.05$) in the duration of proestrus and estrus in the interval from day 10 to day 30 of treatment. The diestrus phase was significantly ($p < 0.01$) reduced compared to the control. The treatment caused continuous maintenance of the estrous cycle of female rats in

estrus. Maintenance of the estrous cycle in estrus was observed in 50% of female rats from day 1 to day 10 of treatment at doses of 50 and 100 mg/Kg body weight. Between days 10 and 20 of treatment with the 50 and 100 mg/kg body weight doses, 75 and 100% of female rats had their estrous cycles maintained in estrus, respectively. From day 20 to day 30, 100% of the animals treated with 50 and 100 mg/Kg body weight had their estrous cycle still maintained in estrus. In contrast, only 33% of rats treated with 200 mg/kg body weight had their estrous cycle maintained in estrus (Table 2).

Table 2. Percentage of animals maintained in estrus.

Treatments in mg/kg bw	Number of rats treated	Percentage of female rats maintained in estrus					
		1 to 10 days		10 to 20 days		20 to 30 days	
		Estrus	Diestrus	Estrus	Diestrus	Estrus	Diestrus
Controls (distilled water)	6	00	00	00	00	00	00
EAPC Dose 50	6	50**	00	75***	00	100***	00
EAPC Dose 100	6	50**	00	100***	00	100***	00
EAPC Dose 200	6	00	00	00	00	33	00

Female rats were treated for the 30 days of treatment with *Petroselinum crispum* aqueous extract. EAPC: Aqueous extract of *Petroselinum crispum*; Data are presented as mean ± Standard error on the mean. Turkey test was used to make comparisons to controls. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

The duration of proestrus was significantly ($p < 0.05$) increased at the 100 mg/Kg body weight dose with a peak of 8.05 ± 0.48 days compared to the control with a peak of 6.5 ± 0.25 days. However, female rats treated with 50 and 100 mg/Kg of body weight with aqueous extract of *P. crispum* leaves showed a highly significant ($p < 0.001$) increase in estrus duration with respective peaks of 28.40 ± 0.48 days and 28.5 ± 0.64 days compared to the control. On the other hand, the 200 mg/Kg body weight dose showed a significant ($p < 0.05$) increase in estrus duration with a peak of 22.00 ± 0.63 days compared to the control group with a peak of 19.20 ± 0.64 days. The duration of diestrus was significantly ($p < 0.01$) decreased by 5.10 ± 0.63; 6.25 ± 0.75 and 11.20 ± 0.63 days at the respective doses of 50; 100 and 200 mg/kg body weight compared to the control group with a duration of diestrus phase of 14.3 ± 0.87 days (Table 3).

Table 3. Effect of EAPC on estrous stages of female rats.

Phases of the Cycle	Pourcentage des rats aux stades œstraux selon les doses			
	Control (distilled water)	EAPC Dose 50	EAPC Dose 100	EAPC Dose 200
Proestrus	6.5 ± 0.25	6.5 ± 0.50	8.05 ± 0.48*	6.80 ± 0.48
Estrus	19.20 ± 0.65	28.40 ± 0.48***	28.50 ± 0.64***	22.00 ± 0.63*
Diestrus	14.30 ± 0.87	5.10 ± 0.63***	3.45 ± 0.75***	11.20 ± 0.63*

Rats were given different doses of aqueous extract of *Petroselinum crispum* daily for 30 days. EAPC: *Petroselinum crispum* Aqueous Extract; Data are presented as mean ± Error on the mean. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ for values without (*) $p > 0.05$; s: significant; ns: not significant. Dose 50: animals treated with the 50 mg/Kg body mass dose of EAPC; Dose 100: animals treated with the 100 mg/Kg body mass dose of EAPC; Dose 200: animals treated with the 200 mg/Kg body mass dose of EAPC.

Fig. 1 A, 1 B, 1 C and 1 D are curves showing the evolution of eosinophilic and leukocytic cells during the treatment of control female rats receiving distilled water and that of female rats treated with 50, 100 and 200 mg/Kg of body weight of CSPA. The daily evolution of the percentages of eosinophilic and leukocytic cells made it possible to appreciate the disturbances of the cycle according to the administered doses. Indeed, in animals receiving distilled water, eosinophilic and leucocytic cells show

a normal evolution for the four phases of the estrous cycle, thus translating the regularity of the cycle. The disturbances are more pronounced with the 50 and 100 mg/kg body weight doses of the aqueous extract of *P. crispum*. They result in an increase in the number of eosinophilic cells, which mark estrus at the expense of leukocytic cells, which characterize diestrus. The abundance of eosinophilic cell peaks is followed by maintenance of the cycle in estrus. The 200 mg/kg body weight dose slightly disrupted the cycle with a significant increase ($p < 0.05$) in eosinophilic cell peaks and a slight significant decrease ($p < 0.05$) in leukocyte cells. The abundance of eosinophilic cell peaks summed up with a brief maintenance in estrus, followed by a rapid relaxation until the end of 30 days of treatment.

Effects of aqueous extract P. crispum on the relative mass of ovaries, uterus horns and adrenal gland

Administration of the aqueous extract of the leaves of *Petroselinum crispum* showed dose-dependent increases in the masses of certain estrogen-dependent organs. The ovary mass of female rats treated at 50 and 100 mg/Kg body weight with aqueous extract of *P. crispum* was 0.092 ± 0.003 and 0.108 ± 0.004 g, respectively. In controls, it is 0.063 ± 0.003 g. The analysis shows a significant ($p < 0.05$) increase in ovarian mass in female rats treated with 50 and 100 mg/Kg body weight of the aqueous extract of *P. crispum*. This increase was 46.03 and 71.43%, respectively, compared with the control group. The uterine horn masses of rats treated with 50, 100 and 200 mg/kg body weight with aqueous extract of *P. crispum* increased very significantly ($p < 0.01$) compared to the control. They are reflected in a percentage of 21.29% for the 200 mg/kg body weight dose and 21.49% for the 50 and 100 mg/kg body weight doses compared to the control. The adrenal gland mass of female rats treated at 50 mg/Kg body weight showed a significant increase ($p < 0.05$) compared to the control (0.020 ± 0.001 g). This increase at 50 mg/Kg body weight was 0.028 ± 0.002 g or 54.64%. However, the adrenal gland mass of female rats treated at 100 and 200 mg/kg body mass showed no significant increase ($p > 0.05$) compared to the control group (Table 4).

Table 4. Effects of *Petroselinum crispum* on the relative masses of estrogen-dependent organs and the adrenal gland.

Treatment	Ovary	Uterus horn	Adrenal gland
Controls	0.063 ± 0.003	0.108 ± 0.005	0.020 ± 0.001
EAPC Dose 50	0.092 ± 0.003**	0.131 ± 0.002**	0.028 ± 0.002*
EAPC Dose 100	0.108 ± 0.004**	0.131 ± 0.002**	0.022 ± 0.003
EAPC Dose 200	0.073 ± 0.004	0.129 ± 0.003**	0.022 ± 0.001

EAPC: Aqueous extract of *Petroselinum crispum*. Data are presented as mean ± Error on Mean. **$p < 0.01$; for values without (*) $p > 0.05$.

Effects of aqueous extract of P. crispum leaves on reproductive hormones

Hormones assay showed that the serum FSH level obtained at 200 mg/kg body weight was significantly ($p < 0.05$) higher than that of control rats and those of female rats treated at 50 and 100 mg/kg body weight. Treatment of female rats with aqueous extract of *P. crispum* leaves at 50 and 100 mg/kg body weight resulted in FSH values below the threshold value (0.055 mIU/ml) compared to the control. However, the control and 200 mg/kg body weight batches showed hormone levels above the threshold value (0.055 mIU/ml) with respective values of 1.35 ± 0.15 and 5.86 ± 0.06 mIU/ml. As for the amount of LH, it was below the threshold in female rats treated with different doses of the aqueous extract.

The analysis showed no significant difference ($p > 0.05$) between the amount of LH of treated female rats and controls. Administration of the aqueous extract of *P. crispum* leaves to the female rats showed a slight non-significant ($p > 0.05$) increase in the level

of estradiol by about 3; 3.74 and 5.49 % respectively at 50, 100 and 200 mg/kg body weight compared to the control group. The determination of progesterone level showed a highly significant increase ($p < 0.01$) in female rats treated with 50 and 100 mg/kg body weight during the treatment with aqueous extract of *P. crispum*. This increase was 17.08 ± 0.91 ng/ml and 34.91 ± 0.92 ng/ml respectively (Table 5).

Table 5. Effect of *Petroselinum crispum* on reproductive hormons.

Treatment	FSH	LH	Estradiol	Progesterone
Controls	1.35 ± 0.15	1.17 ± 0.05	66.67 ± 0.69	9.49 ± 0.745
EAPC Dose 50	< 1	< 1	69.17 ± 0.44	17.08 ± 0.92**
EAPC Dose 100	< 1	< 1	68.67 ± 0.88	34.91 ± 0.92***
EAPC Dose 200	5.86 ± 0.06*	< 1	70.33 ± 0.88	9.63 ± 0.69

Female rats received daily for 30 days different doses of aqueous extract of *Petroselinum crispum*. EAPC: Aqueous extract of *Petroselinum crispum*. Data are presented as mean ± Error on Mean. **$p < 0.01$; ***$p < 0.001$ for values without (*) $p > 0.05$; Dose 50: animals treated with the 50 mg/Kg dose of EAPC body mass; Dose 100: animals treated with the 100 mg/Kg dose of EAPC body mass; Dose 200: animals treated with the 200 mg/Kg dose of EAPC body mass.

Female rats received daily for 30 days different doses of aqueous extract of *Petroselinum crispum*. EAPC: Aqueous extract of *Petroselinum crispum*. Data are presented as mean ± Error on Mean. **$p < 0.01$; ***$p < 0.001$ for values without (*) $p > 0.05$; Dose 50: animals treated with the 50 mg/Kg dose of EAPC body mass; Dose 100: animals treated with the 100 mg/Kg dose of EAPC body mass; Dose 200: animals treated with the 200 mg/Kg dose of EAPC body mass.

Discussion

The qualitative phytochemical analysis of the aqueous extract of the leaves of *Petroselinum crispum* allowed to highlight the presence of flavonoids, polyphenols, tannins, saponosides, sterols, terpenes and alkaloids. The richness of this plant in secondary metabolites would explain its use in traditional pharmacopoeia, especially in the treatment of infertility.

These results are similar to those of Ozsoy-Sacan *et al.* (2006) in the use of *P. crispum* extract. Similar results were obtained in ethnopharmacology, phytochemistry and biological activities studies of *P. crispum* (Farzaei *et al.*, 2013). The phytochemistry of *P. crispum* is similar to other plants used in African pharmacopoeia Kouakou and Tahiri (2018). Some studies have shown that flavonoids and alkaloids have estrogenic, hepatoprotective, anti-inflammatory properties. Terpenoids are potentially endowed with anti-inflammatory and sometimes analgesic properties (Bennett *et al.*, 2003).

The estrous cycle is responsible for the cornification of the vaginal mucosal cells leading to the proestrus and estrus phases (Russell, 2008). The different vaginal smears performed daily on female rats at the three doses of the aqueous extract of *P. crispum* leaves showed an increase in the number of proestrus and estrus peaks at the expense of the diestrus phases at the doses of 50 and 100 mg/kg body weight. Indeed, 100% of female rats treated with aqueous extract of *P. crispum* had their estrous cycle maintained in estrus from day 20 to day 30 of treatment at doses 50 and 100 mg/kg body weight. But the 200 mg/Kg body weight dose showed a 33% increase. This significant increase ($p < 0.01$) in the duration of the estrus phase could be explained by the maturation of the follicles in the ovary. However, the total duration of estrus decreased significantly ($p < 0.01$) during the 30 days of treatment for the doses of 50, 100 and 200 mg/Kg of body mass. These observed effects are similar to those obtained when administering the aqueous extract of *Moringa oleifera* leaves, which resulted in an extension of estrus duration, as well as maintenance of this phase in treated female rats (Kouakou *et al.*, 2018).

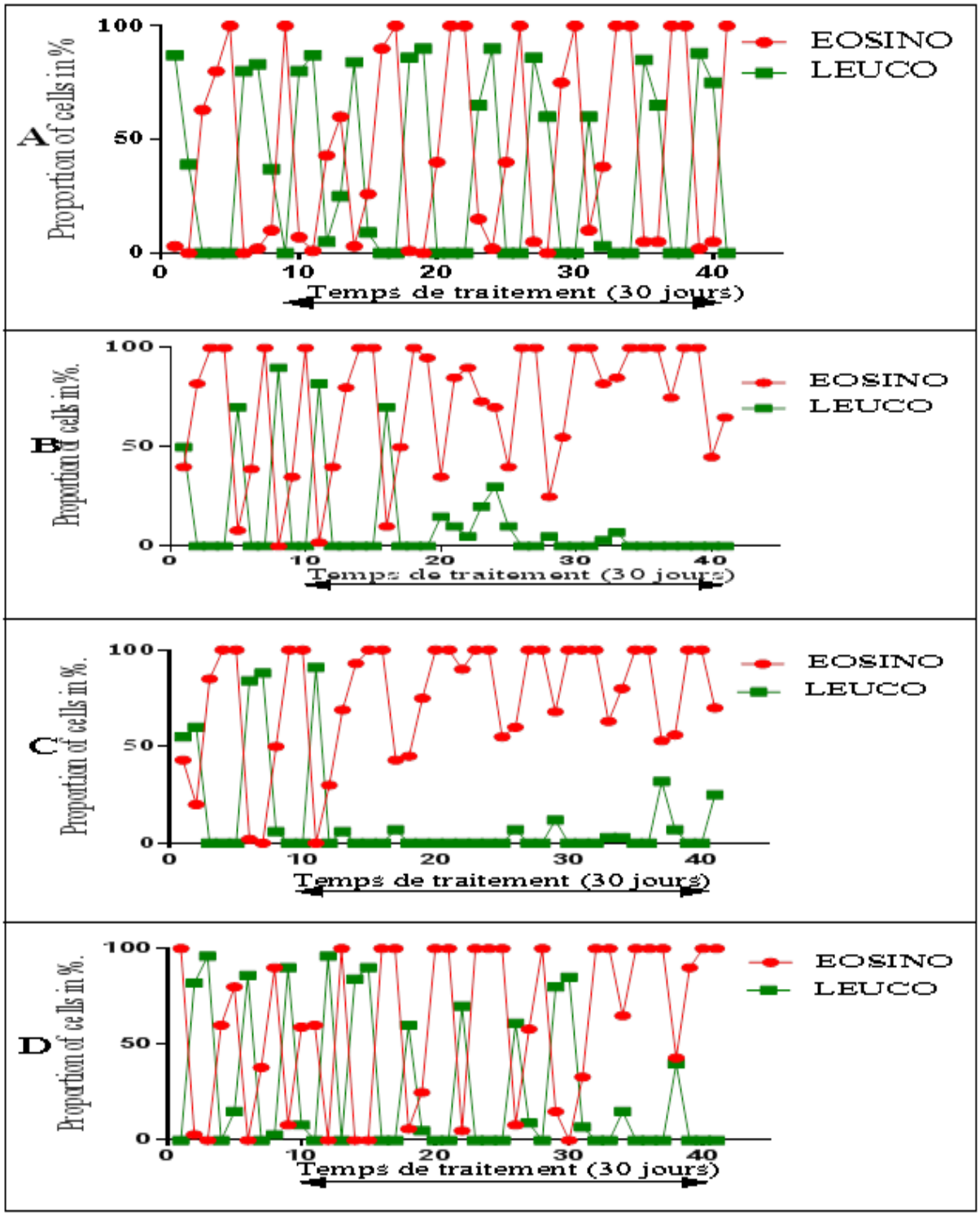

Fig. 1. Evolution of the estrous cycle of female rats treated with *Petroselinum crispum* A: Control female rat (distilled water: no blockage); B: Female rat treated with 50 mg/kg bw; C: Female rat treated with 100 mg/kg bw; D: Female rat treated with 200 mg/kg bw. The arrow (⟷) indicates the start and end of the treatment.

The increase in the duration of the estrus phase at doses 50 and 100 mg/kg body weight, would be due to the presence of estrogen agonist substance. Indeed, the estrogen-like substances contained in *P. crispum* extracts could act by a direct action on the vaginal cells or on the ovary. They would also act indirectly on the hypothalamo-hypophyseal complex through the release of gonadotropic hormones (Hu and Aizawa, 2003). Other authors have also shown similar results by observing the increase in the duration of proestrus

and estrus by the administration of ethanolic extract of *Rhynchosia sublobata* at the dose 2500, 5000 and 1000 mg/Kg of body mass to female rats (Mustapha *et al*., 2011). The aqueous extract of *P. crispum* leaves would have a similar action to 17-β-estradiol. Thus Kouakou *et al.* (2018) and Affy *et al.* (2019) demonstrated that repeated administration of 17-β-estradiol at the respective dose of 20.10-3 mg/kg body weight to adult female rats prolongs the estrous cycle at the estrus stage. The prolongation of the duration of proestrus and estrus could also be explained by the presence of tannins, flavonoids and saponosides revealed during the phytochemical screening. These effects could be due to the presence of molecules such as alkaloids, flavonoids, saponosides and sterols that are known to have estrogenic effects (Rimoldi *et al*., 2007). These results support observations that the administration of certain xenobiotic substances can alter the cycle by lengthening or reducing specific stages (Bleu *et al*., 2012).

The mass of the ovaries of female rats treated with 50 and 100 mg/ Kg body weight of the aqueous extract of *P. crispum* leaves increased very significantly compared to control rats. Indeed, during the estrous cycle, the follicles evolve into De Graaf follicles by increasing their size, the number of cell layers (granulosa, external and internal theca) and their fluid cavity (Gayrard, 2007). The increase in ovarian mass of female rats treated with aqueous extract of *P. crispum* at doses of 50 and 100 mg/kg body weight are similar to those of Bleu *et al.* (2012), who observed an increase in ovarian mass after administration of *Passiflora foetida*. This increase could be explained by high ovarian activity in treated animals compared to controls. Indeed, during the estrous cycle of the female rat, the ovary is marked by two major phases, namely a follicular phase and a luteal phase. The follicular phase is characterized by a growth of the follicles until the De Graff follicle is reached and production of estradiol which could induce an increase in the mass of the ovary (Monniaux *et al*., 2009). The maximum of these phenomena is reached in the proestrus phase when the gonadotropin level is at its maximum and resulting in an increase in ovarian mass (Haim *et al*., 2003). Other authors have shown that administration of aqueous extract of Anethum graveolens at doses of 200, 400, 600 and 800 mg/Kg of body mass resulted in no increase in ovarian mass of treated female rats compared to control groups (Raji *et al*., 2012). The change in ovarian mass would be induced by low doses, according to its authors.

The uterine horn mass of rats treated at 50, 100 and 200 mg/Kg body weight with aqueous extract of *P. crispum* leaves was significantly increased compared to the control. The increase in uterine horn weight of treated rats would be due to an abundance of fluid in the uterine horn. This increase would confirm that the aqueous extract of *P. crispum* leaves could contain estrogen-like substances. Indeed, some authors have observed an increase in uterine horn mass following the administration of Artemisia vulgaris leaf extract to female rats (Shaik *et al*., 2014). Indeed, the increase in uterine horn mass confirms that the aqueous extract of *P. crispum* leaves would be the source of protein synthesis. Also, the steroids, saponosides and flavonoids contained in the aqueous extract would exert estrogenic effects on the central nervous system (Adaay *et al.,* 2013). The aqueous extract of *P. crispum* leaves reported to contain estrogenic substances or phytoestrogens that may stimulate ovarian activity in female rats. Müller *et al.* (2009) showed that the binding of phytoestrogens to the receptors of the uterine horn produces a chain of reactions allowing the biosynthesis of macromolecules which would cause the increase of its mass.

The dose of 50 mg/kg body weight of the aqueous extract of *P. crispum* leaves caused a significant ($p < 0.05$) increase in the mass of the adrenal gland. This result might suggest that the aqueous extract of *P. crispum* at the dose of 50 mg/Kg body mass causes the production of adrenocorticotropin and, therefore, would act to increase the adrenal gland mass. The protein and steroid hormones allow a better appreciation of the pharmacological effect of the

extracts. The LH level of female rats treated with the different doses did not show any significant variation compared to the control of female rats. In contrast, the estradiol level showed a slight increase compared to the control. This slight increase in estradiol in this study may confirm the observed increase in ovarian and uterine horn mass. These results would therefore confirm the estrogenic character of the aqueous extract of *P. crispum* leaves. These results seem to corroborate those of some authors on the increase over time (7, 14, 21 and 28 days) in plasma estradiol levels induced by *Hibiscus sabdariffa* extract (Sirag *et al.,* 2013). Progesterone is a steroid hormone that modulates the proliferative activity of estradiol in the endometrium and is essential for implantation of the blastocyst and then the maintenance of gestation (Perrot-Applanat, 1997). It is secreted by the corpus luteum in the ovary in the second part of the sexual cycle. Progesterone was significantly increased at 50 and 100 mg/kg body weight compared to the control. Indeed, during the luteal phase, the concentration of progesterone is higher than estradiol. The elevation of progesterone level prevents any positive feedback of estradiol necessary to trigger the LH peak and, therefore, ovulation. This would explain the lack of change in LH of treated female rats compared to controls. The significant increase ($p < 0.05$) in progesterone levels could be due to the estrogenic effect of the aqueous extract of *P. crispum* leaves. Indeed, *P. crispum* contains alkaloids and flavonoids, which are substances known for their estrogenic effects (Yakubu *et al.*, 2011).

Conclusion

In female rats, the aqueous extract of *Petroselinum crispum* leaves maintains the estrous cycle in estrus between the 20th and 30th day of treatment. The aqueous extract of the leaves of *P. crispum* significantly increases the relative mass of the reproductive organs as well as the concentration of the sex steroids estradiol and progesterone. The extract also stimulates follicular development and ovulation while inducing the estrus phenomenon at low doses. On the other hand, the aqueous extract of *P. crispum* has no action on the secretion of LH.

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