INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 23, No. 6, p. 196-207, 2023

RESEARCH PAPER OPEN ACCESS

Uterotrophic activities of the aqueous extract of *Moringa oleifera* (Moringaceae) Lam. 1785 on young ovariectomized rats

Kouakou Koffi Roger*[1], Affy Mataphouet Emmanuel Guy Jocelin[1], Kablan Kassi Jean-Jacques[2], Tahiri Annick[2]

[1]*Université Alassane Ouattara, UFR Sciences Médicales, Département des Sciences et Techniques, Bouaké, Côte d'Ivoire*

[2]*Université Félix Houphouët-Boigny, UFR Biosciences, Laboratoire de Biologie et Santé, Unité Pédagogique et de Recherche de Reproduction et Développement Animal, Abidjan, Côte d'Ivoire*

Key words: Rats, Ovariectomy, Uterus, Trophic uterus, Adrenal glands

http://dx.doi.org/10.12692/ijb/23.6.196-207 Article published on December 12, 2023

Abstract

Studies have been carried out to determine the estrogenic activity of *Moringa oleifera*. However, less data exists on the uterotrophic effect of the extract of this plant. This study aims to evaluate the uterotrophic effects of the aqueous extract of *Moringa oleifera* in young ovariectomized rats. The plant material consists of *Moringa oleifera* leaves and the animal material consists of Rattus norvegicus rats. Young rats underwent bilateral ovariectomy according to the guideline of the Organization for Economic Co-operation and Development. Batch 1 received distilled water. The animals of batches 2, 3, 4 and 5 received respectively olive oil, 17β-estradiol at 2.10^{-2} mg/Kg of body weight, doses of 150 and 300 mg/Kg of body weight of aqueous extract of *Moringa oleifera*. Lots 6 and 7 respectively received doses of 150 and 300 mg/Kg body weight of extract, each associated with a dose of 2.10^{-2} mg/Kg body weight of 17β-estradiol. The animals were weighed during the seven days of treatment. The fresh and dry weights of rats experienced a significant increase ($p<0.05$) only with 17β-estradiol at 2.10^{-2} mg/Kg bw, doses of 150 and 300 mg/Kg bw of aqueous extract of *M. oleifera* each associated with the dose of 2.10^{-2} mg/Kg of body weight of 17β-estradiol compared ($p<0.05$) to those of the respective controls. *Moringa oleifera* appears to have uterotrophic effects on young ovariectomized rats after seven days of treatment.

* **Corresponding Author:** Kouakou Koffi Roger ✉ kouakoukoffiroger2016@gmail.com

Introduction

Ivory Coast is a country with high floral diversity. Of the nearly 1,500 species of plants identified in Ivory Coast, 1,421 are used in traditional medicine and provide patient care (Aké-Assi, 2002). This work has highlighted biocompounds with diversified pharmacological properties, phytochemical properties and toxic properties of plants from traditional Ivorian medicine (Touré, 2015). Some work has shown that plants containing phytoestrogens were capable of improving the fertility of mammals (Bleu, 2012; Blahi, 2017; Zougrou, 2018). *Moringa oleifera* is one of 240 plants from 120 species studied in the context of viral pathologies. This Moringaceae from the Ivorian pharmacopoeia appears to be one of the most promising species, given its nutrient content and its phytochemical compounds (Yang *et al.*, 2006). These assets have allowed researchers to study its immuno-modulatory (Shaila *et al.*, 2010) and hepatoprotective (Panda *et al.*, 2013), anti-tumor (Purwal *et al.*, 2010) and estrogenic properties (Kouakou *et al.*, 2018) and in the treatment of many other pathologies. However, these utero-trophic properties are less elucidated. The objective of this work is to evaluate the utero-trophic activity of the aqueous extract of *Moringa oleifera* on young ovariectomized rats.

Materials and methods

Plant material

The leaves of *M. oleifera* were harvested during the month of June in the Abidjan District, in the south of Côte d'Ivoire. A sample of this plant was identified at the National Floristic Center (CNF) of the Felix Houphouët-Boigny University under the numbers 8685 and 14533. The choice was made on the leaves of this plant, because they are consumed by the Ivorian population in various forms. They are also used traditionally not only for the treatment of infertility cases, but also for cases of termination of pregnancy (Philippi, 1997).

Animal material

The animal material used for the present study consists of 42 young rats of the species Rattus norvegicus (Muridae) of the Wistar strain. These rats are six weeks old and weigh between 60 and 70 grams. These animals come from the vivarium of the Ecole Normale Supérieure (ENS) in Abidjan. The animals were fed daily add libitum. The choice of this animal species was based on its availability and its high use in pharmacology.

Preparation of the aqueous extract of M. oleifera

The harvested leaves are rinsed thoroughly with tap water. They were dried away from the sun and at room temperature (30 ± 2°C) for two weeks. The dried leaves are pulverized using an electric crusher of the IAMAG-RCT® type. A 50 g portion of this powder was macerated in 1.25 L of distilled water for five times three minutes in a blender (Single®, Singapore). The macerate gave a 4% stock solution. The macerate was filtered three times on white cloth then successively on hydrophilic cotton and on Wattman No. 1 paper. The filtrate obtained was evaporated in an oven at 50°C for 48 h. The brown dry extract was used for the various tests (Zirihi *et al.*, 2003).

Ovariectomy technique for young rats

The proposed technique is bilateral oophorectomy. Bilateral oophorectomy was performed on the animal anesthetized and lying on its stomach. After shaving and disinfection with alcohol, the dorsolateral abdominal wall was incised approximately one centimeter. This incision was made exactly at the midpoint between the lower costal limit and the iliac crest, a few millimeters from the lateral margin of the lumbar muscle. The ovaries were thus extracted from the abdominal cavity, placed on an aseptic field then detached at the junction of the oviduct and the uterine body. After checking that no significant bleeding occurred, the abdominal wall was sewn up with 2/0 diameter (absorbable) sutures. The skin was also closed by suturing with a 2/0 diameter suture thread (nylon). Painkillers were administered to animals to reduce post-surgical discomfort (OECD, 2008).

Selection of young ovariectomized rats by vaginal smears

In order to allow regression of the uterus to a stable minimum reference weight, a time interval of 14 days was left between the ovariectomy of six-week-old rats

and the first administration of the substances. To prevent a small amount of ovarian tissue from causing an increase in circulating estrogen, animals were selected before the test by observing the epithelial cells. This observation was carried out on each ovariectomized rat for five consecutive days (from the 10th to the 14th day after ovariectomy) through a vaginal smear. Animals that showed signs of proestrus and estrus were immediately removed from testing.

Formulation of ethinyl estradiol solution

Estradiol ethinyl served as a reference for the uterotrophic study of the aqueous extract of *M. oleifera*. The tablets (50 µg estradiol/tablet) were gently ground. The powder obtained was mixed with olive oil. The whole thing was carefully homogenized using a spatula. A concentration of 2.10^{-2} mg/Kg of body weight was taken in a volume of 1 ml of olive oil (extra virgin).

Administration protocol for different substances

The selected ovariectomized rats were divided into seven groups of six animals. Each batch was treated for seven days with different doses (Blahi, 2017). Batch 1 received distilled water. Then, the animals of batches 2, 3, 4 and 5 received respectively olive oil, 17β-estradiol at 2.10^{-2} mg/Kg of body weight, doses of 150 and 300 mg/Kg of body weight of aqueous extract of *M. oleifera*. Lots 6 and 7 respectively received doses of 150 and 300 mg/Kg bw of aqueous extract of *M. oleifera* each associated with a dose of 2.10^{-2} mg/Kg bw of 17β-estradiol. The rats were weighed every day at the same time during the seven days of treatment. The variation in the weight of the rats was calculated according to the formula below:

Weight variation (%) = (Initial weight/Final weight) ×100

Effect of the aqueous extract of M. oleifera on the weight of the uterine horns and adrenal glands of young ovariectomized rats

On the 7th day of treatment and 24 h after administration of the last dose, the animals from each batch were sacrificed by decapitation after ether anesthesia. The organs, which are the uterine horns and the right adrenal glands, were removed by opening the abdominal cavity. These organs were rinsed in 0.9% NaCl, dried on paper towels, and weighed for fresh weight. The organs were subsequently dried in an oven at 100°C for 24 h and weighed again to determine the dry weight. The removed uterine horn and left adrenal gland were preserved in 10% formalin.

To identify estrogen agonists, measurements were made to establish the ratio between the average uterine weight of animals from treated groups and that of animals from control groups which received distilled water. A statistically significant increase in the average uterine weight of a test group indicates a positive response of the uterotrophic bioassay. The relative weight of the uterine horn was determined according to the formula:

Relative weight (g/100g de bw)=(Organ weight/Body weight)×100

Effect of the aqueous extract of M. oleifera on the histology of the uterine horns and adrenal glands of young ovariectomized rats

Preserved in 10% formalin, the left uterine horn and adrenal gland were used for histological study. This study aims to assess the general architecture of these organs, the height of the epithelial cells, the thickness of the endometrium and the number of uterine glands. This technique follows several steps (Hould, 1984). These steps are fixation, alcohol dehydration and clearing, impregnation, paraffin embedding, microtome sectioning, deparaffinization of organs, staining of organ sections, mounting and observation

Effect of M. oleifera extract on the morphometric parameters of the uterine horns of young ovariectomized rats

From the histological sections of the uterine horns, morphometric parameters such as endometrial thickness and number of uterine glands were measured as indicated by Sripriya *et al.* (2011).

Statistical analysis

Statistical analyzes were performed using Graph Pad Prism 5.0 software. Values were presented as means ± Standard Error of the Mean (SEM). The analysis of

variance (ANOVA) applied to the results obtained made it possible to assess the effects of the different treatments with a significance level of 5%. The Turkey-Kramer multiple comparison test was used to make comparisons between means.

Results

Effects of the aqueous extract of M. oleifera on the body weights of young ovariectomized rats

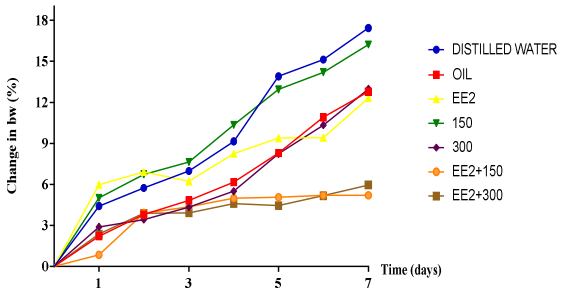

Fig 1. Effects of *Moringa oleifera* aqueous extract on body weights of ovariectomized immature rats

Test substances were administered to different batches of young ovariectomized rats daily for seven days; 150: 150 mg/Kg bw of aqueous extract of *M. oleifera*; 300: 300 mg/Kg bw of aqueous extract of *M. oleifera*; EE2: Ethinyl estradiol (2.10-3mg/Kg bw); EE2+150 mg/Kg bw: Estradiol ethinyl (2.10-3mg/Kg bw) + 150 mg/Kg bw of aqueous extract of *M. oleifera*; EE2+300 mg/Kg bw: Estradiol ethinyl (2.10-3mg/Kg bw) + 150 mg/Kg bw of aqueous extract of *M. oleifera*. Data were presented as mean ± Error of the mean (ESM) (n = 6/batch). The Turkey test was used to make comparisons against controls

For seven days, the administration by gavage of distilled water, olive oil, ethinyl estradiol and aqueous extract of *M. oleifera* to young ovariectomized rats made it possible to assess the variation in the body mass of rats (Fig. 1). Indeed, the weight masses of animals treated with ethinyl estradiol alone and ethinyl estradiol associated with doses of 150 and 300 mg/Kg bw of aqueous extract of *M. oleifera* decreased significantly ($p < 0.01$), respectively 12.02 ± 0.1114; 6.871±0.5296 and 6.867±0.3208% compared to those of controls treated with distilled water (15.28±1.935%). But, the weight masses of the animals treated only with the aqueous extract of M. *oleifera* at doses of 150 and 300 mg/Kg bw with respective values of 15 ± 0.7118 and 12.38 ± 0.7373% did not have not significantly ($p > 0.05$) elevated compared to those of controls treated with distilled water (15.28 ± 1.935%).

Effects of the aqueous extract of M. oleifera on the vaginal opening and the presence of epithelial cells in young ovariectomized rats

Estradiol administered alone for seven days caused vaginal opening in all rats (100%). This value was significantly high ($p < 0.05$) compared to those of rats treated with doses of 150 and 300 mg/kg bw of aqueous extract of *M. oleifera* which showed respectively 40 and 60% openings vaginal. They were also significantly elevated ($p < 0.05$) compared to those of the controls who received distilled water. These opening percentages increased significantly ($p < 0.05$) by 80 and 100% after the respective combination of estradiol + 150 mg/kg bw *M. oleifera* and estradiol + 300 mg/kg bw of aqueous extract of *M. oleifera* compared to those of rats treated only at doses of 150 and 300 mg/kg bw of aqueous extract of *M. oleifera* (Table 1).

Table 1. Effects of test substance treatments on the vaginal opening and the presence of epithelial cells in the genital tract.

Treatment of animals	Percentage vaginal opening (%)	Presence of epithelial cells
Controls (distilled water)	00	-
Olive oil control	00	-
Estradiol	100	++
AEMO (150 mg/Kg bw)	40	+
AEMO (300 mg/Kg bw)	60	+
Estradiol + AEMO (150 mg/Kg bw)	80	++
Estradiol + AEMO (300 mg/Kg bw)	100	++

Data were presented as mean ± Error of the mean (ESM) (n = 6/batch). The Turkey test was used to make comparisons against the Controls. AEMO: Aqueous Extract of *M. oleifera*; +: Indicates the presence of epithelial cells; ++: Indicates a strong presence of epithelial cells; -: Indicates an absence of epithelial cells. The comparison of the table is made in the vertical direction.

Table 1 also shows the results of vaginal smears taken from ovariectomized rats on the seventh day of treatment. Indeed, smears taken from rats treated with estradiol alone revealed an abundance of eosinophil cells. The combinations of estradiol + 150 mg/kg bw of *M. oleifera* extract and estradiol + 300 mg/kg bw of aqueous extract of *M. oleifera* revealed a very significantly ($p < 0.05$) high number of eosinophil cells compared to controls (distilled water and olive oil). Doses of 150 and 300 mg/kg bw of *M. oleifera* extract administered to rats resulted in a low presence of eosinophil cells compared to those treated with estradiol and the combination of estradiol + *M. oleifera* extract. But the number of cells obtained only with doses of extract remains higher than that obtained with distilled water and oil.

Effects of the aqueous extract of M. oleifera on the weights of the uterine horns and the adrenal gland in young ovariectomized rats

The effect of the aqueous extract of *M. oleifera* was evaluated on the fresh and dry weights of the uterine horns of young ovariectomized rats (Fig 2). Indeed, the fresh weights of the uterine horns were 0.0110 ± 0.0003 g and 0.0116 ± 0.0004 g for the ovariectomized rats having received the aqueous extract of *M. oleifera* at the respective doses of 150 and 300 mg/Kg bw. These values showed no significant change ($p > 0.05$) when compared to those of controls treated with distilled water (0.0128 ± 0.0004 g). But, these values were very significantly low ($p < 0.01$) compared to those of the positive controls treated with 17 β-estradiol (0.0298 ± 0.0007 g). Only the fresh and dry weights of the uterine horns of rats having undergone treatments with 17 ß-estradiol + 300 mg/Kg bw of aqueous extract of *M. oleifera* were significantly elevated ($p < 0.05$) compared to controls (distilled water, 17 ß-estradiol alone and olive oils).

The fresh and dry weights of the adrenal glands were also evaluated during this study (Fig 3). Thus, the fresh and dry weights of the adrenal glands of rats treated with the aqueous extract of *M. oleifera* alone at doses 150 and 300 mg/kg bw, with the combination of estradiol + 150 mg/kg bw extract of *M. oleifera* and estradiol + 300 mg/kg bw of aqueous extract of *M. oleifera* did not differ significantly ($p > 0.05$) compared to those of rats treated with distilled water and compared to those of rats treated with 17 β-estradiol alone.

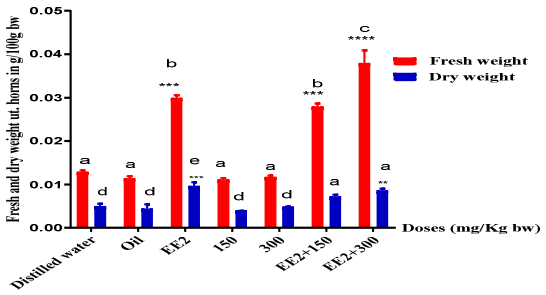

Fig 2. Effects of aqueous extract of *Moringa oleifera* on the fresh and dry weight of the uterine horns of ovariectomized immature rats

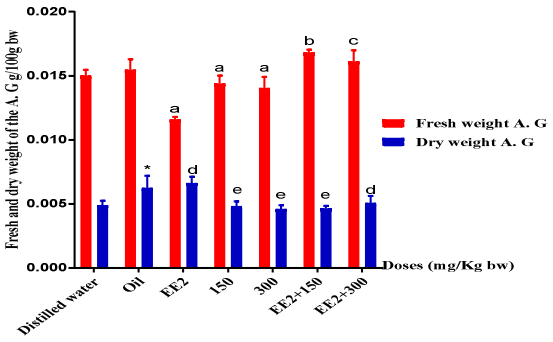

Fig 3. Effects of the aqueous extract of *Moringa oleifera* on the fresh and dry weight of the adrenal glands of ovariectomized immature rats

ut: Uterine; A G: Adrenal Gland; 150: 150 mg/Kg bw of aqueous extract of *M. oleifera*; 300: 300 mg/Kg bw of aqueous extract of *M. oleifera*; EE2: Ethinyl estradiol (2.10-3mg/Kg bw); EE2+150 mg/Kg bw: Estradiol ethinyl (2.10-3mg/Kg bw) + 150 mg/Kg bw of aqueous extract of *M. oleifera*; EE2+300 mg/Kg bw: Estradiol ethinyl (2.10-3mg/Kg bw) + 150 mg/Kg bw of aqueous extract of *M. oleifera*. Data were presented as mean ± Error of the mean (ESM) (n = 6/batch). The Turkey test was used to make comparisons against the Controls.

Effects of the aqueous extract of M. oleifera on the histology of the uterine horns and adrenal glands in young ovariectomized rats.

At the end of daily administration (for seven days) of *M. oleifera* extract at doses of 150 and 300 mg/kg bw,

the number of uterine glands was 31 ± 10.7 and 32 ± 12, respectively. This number does not reveal any significant increase ($p > 0.05$) compared to rats treated with distilled water (25.3 ± 8.8).

Estradiol treatment of rats resulted in a highly significant increase ($p < 0.001$) in the number of uterine glands (38.10 ± 7 or 52%) compared to the control (25.3 ± 8.8). The effect of the combination of estradiol and 150 and 300 mg/Kg bw *M. oleifera* presented a very highly significant increase ($p < 0.001$) in the number of uterine glands (43.6 ± 7.4 and 49 ± 8.5, an increase of 72.33 and 93.44%) compared to rats treated with distilled water and estradiol alone (Table 2).

Table 2. Effects of aqueous extract of Moringa oleifera and 17 ß-estradiol on uterine morphometric parameters of ovariectomized rats

Treatment of animals	Number of uterine glands	Endometrial thickness (µm)
Controls (distilled water)	25,3 ± 8,8	108,3 ± 6,6
Estradiol	38,0 ± 7***	206,7 ± 4,2****
EAMO (150 mg/Kg bw)	31 ± 11,7	132,7 ± 5,0
EAMO (300 mg/Kg bw)	32 ± 12,5	135,7 ± 5,2
EAMO (150 mg/Kg bw) + Estradiol	43,67 ± 7,4***	228,3 ± 6,0****
EAMO (300 mg/Kg de pc) + Œstradiol	49 ± 8,5***	265, 0 ± 13,3***

Data were presented as average (n = 6/batch); The Turkey test was used to make comparisons against the Controls. AEMO: Aqueous Extract of *M. oleifera* *: $p < 0.05$; **: $p < 0.01$); ***: $p < 0.001$); ***: $p < 0.0001$); for values without (*), $p > 0.05$. The comparison of the table is made in the vertical direction

The thickness of the endometrium of rats treated with the aqueous extract of *M. oleifera* at doses of 150 and 300 mg/kg bw was 132.7 ± 5.0 µm and 135.7 ± 5.2, respectively. These values reflect a non-significant increase rate ($p > 0.05$) of 22 and 25% respectively compared to the control treated with distilled water (108.3 ± 6.6 µm). In rats treated with 17 ß-estradiol, the thickness of the endometrium presented a diameter of 206.7 ± 4.2 µm, a highly significant increase ($p < 0.001$) of 90.85% compared to that treated with distilled water.

The effect of estradiol associated with each dose of 150 and 300 mg/Kg bw of aqueous extract of *M. oleifera* gave diameters of 228.3 ± 6.0 µm and 265.0 ± 13, respectively. 3 µm. These figures indicate a very highly significant increase ($p < 0.0001$) of 110 and 144% respectively in the increase in endometrial thickness compared to the animals treated with distilled water (Table 2).

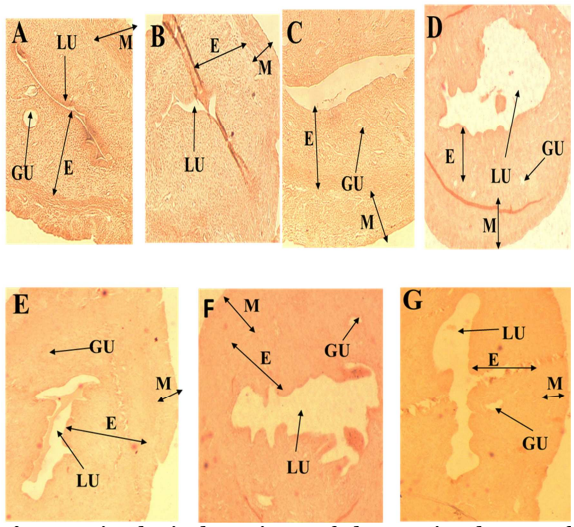

Fig 4. Histological sections of the uterine horns of ovariectomized immature rats treated with aqueous extract of *M. oleifera*.

Coloring: Hematoxylin and Eosin; Gx40, Gx100. Rats were treated daily with repeated doses of the aqueous extract of *M. oleifera* for seven days. LU: Uterine Light; M: Myometrium; In: Endometrium; GU: Uterine gland; **A**: Rats Treated with distilled water (Hematoxylin and eosin, G x 100); **B**: Rats Treated with Olive Oil (Hematoxylin and eosin, G x 100); **C:** Rats Treated with 150 mg/Kg bw of aqueous extract of *M. oleifera* (Hematoxylin and eosin, G x 100); **D:** Rats Treated with 300 mg/kg body weight of aqueous extract of *M. oleifera* (Hematoxylin and eosin, G x 40); **E**: Rats Treated with Estradiol (Hematoxylin and eosin, G x 40); **F**: Rats Treated with 150 mg/Kg bw of aqueous extract of *M. oleifera* + Estradiol (Hematoxylin and eosin, G x 40); G: Rats Treated with 300 mg/Kg bw of aqueous extract of *M. oleifera* + Estradiol (Hematoxylin and eosin, G x 40).

Micrographs of the uterine horns (Fig. 4) demonstrated uterine lumen, myometrium, endometrium, and uterine glands. The micrographs of the adrenal glands (Fig. 5) showed, on the one hand, the adrenal cortex or adrenal cortex formed from the outside towards the inside of a glomerulosa layer, a fasciculate layer and a reticular layer and on the other hand, the adrenal medulla or adrenal medulla located in the center of the adrenal gland.

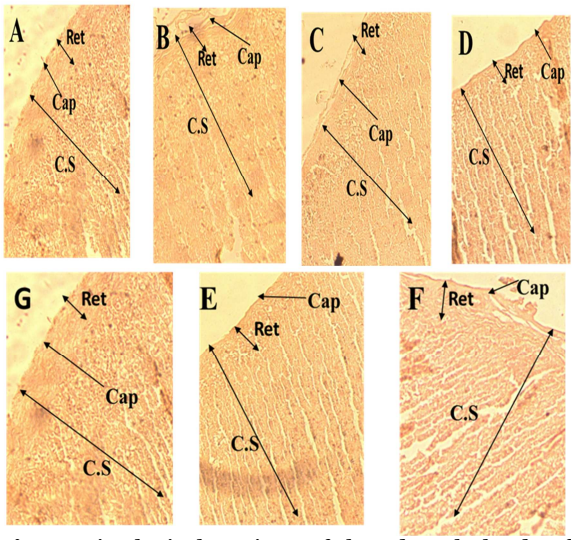

Fig 5. Histological sections of the adrenal glands of ovariectomized immature rats treated with aqueous extract of *M. oleifera*

Coloring: Hematoxylin and Eosin; G x 40, G x 100. Rats were treated daily with repeated doses of the aqueous extract of *M. oleifera* for seven days. CS: Adrenal cortex; Cap: capsule; Ret: reticulated area. A: Rats Treated with distilled water; B: Rats Treated with Olive Oil; C: Rats Treated with 150 mg/Kg bw of aqueous extract of M. oleifera; D: Rats Treated with 300 mg/kg body weight of aqueous extract of M. oleifera; E: Estradiol Treated Rats; F: Rats Treated with 150 mg/Kg body weight of aqueous extract of M. oleifera + Estradiol; G: Rats Treated with 300 mg/Kg bw of aqueous extract of M. oleifera + Estradiol

Discussion

Estrogen appears to be an essential requirement for the growth and differentiation of the uterus, uterine horn and vagina (Jensen and DeSombre, 1972). The estrogenic effect of the aqueous extract of *M. oleifera* was carried out using a uterotrophic bioassay (Dorfman, 1962). This trial made it possible to describe the positive influence of the aqueous extract of *M. oleifera* on the growth of uterine tissues.

The evaluation of the body weight of ovariectomized immature rats treated with the aqueous extract of *M. oleifera* at doses of 150 and 300 mg/Kg bw for seven days was carried out. No significant difference ($p < 0.05$) in the body weights of rats treated at doses of 150 and 300 mg/kg bw was observed compared to those of the controls. On the other hand, the body weight of rats treated with 17β-estradiol at 2.10^{-2} mg/Kg bw were significantly ($p < 0.05$) low compared to that of controls (distilled water). The 17β-estradiol association at 150 and 300 mg/Kg body weight of *M. oleifera* extract separately did not significantly influence the weight of ovariectomized rats compared to the control (distilled water). These results could suggest that the treatment time (seven days), at the dose of 150 and 300 mg/kg body weight of extract would not be sufficient enough to induce the estrogenic effect in order to reduce or maintain the weight of ovariectomized rats. The increase in weight of the controls could be explained through the progressive accumulation of abdominal fat and the increase in dyslipidemia in these ovariectomized rats (Cekmez *et al.*, 2011). The study by Zoth *et al.* (2010) on Wistar rats indicates that ovariectomy increases the susceptibility of developing obesity.

Some authors have reported that estrogen decreases lipogenesis and food intake through central nervous control over the feeling of satiety (Butera, 2010). The study by Saengsirisuwan *et al.* (2009) showed that ovariectomized animals had a higher final body weight, fat and a significantly elevated LDL level ($p < 0.05$) compared to those of sham animals (non-ovariectomized). The administration of estradiol, during this study, significantly reduced body weight by 25%, visceral fat by 51%, and LDL by 26%. Blahi (2017) also showed that the body weight of ovariectomized immature rats treated with *Sarcocephalus latifolius* extract at different doses was proportional to that of those treated with 17ß-estradiol at 2.10-3 mg/Kg bw.

Results contrary to the effect of the aqueous extract of *M. oleifera* were obtained by Kouakou (2007). Indeed, according to the work of this author, treatments of ovariectomized rats with extracts of *Arachis hypogaea* and *Phaseolus vulgaris* accentuated the increase in weight which was respectively 17% ($p < 0.05$) and 18%. ($p < 0.05$) compared to that of the control group (28%).

The extract does not significantly influence the vaginal opening, the epithelial cells of the vagina, the fresh and dry weight of the uterine horns at doses of 150 and 300 mg/Kg bw compared to the controls. However, in those treated with 17β-estradiol alone and 300 mg/kg bw of *M. oleifera* extract, a high number of rats with vaginal opening, vaginal epithelial cells and weight values of fresh uterine horns was noted. and dry significantly ($p < 0.05$) elevated compared to those of the distilled water controls. However, these values remained significantly ($p < 0.05$) low compared to the results obtained with the combination of 17β-estradiol + 150 mg/Kg of body weight and 17 β-estradiol + 300 mg/Kg of body weight.The overall effects of administration of the aqueous extract of *M. oleifera* were also found to be dependent on the administered dose. At a low dose (150 mg/kg of extract), practically no notable effects were noted. When the dose was doubled (300 mg/Kg of extract), a rapid effect of the extract on these same parameters was obtained. The effect was more marked by the association of different doses of aqueous extracts of *M. oleifera* (150 and 300 mg/Kg bw) with 17 β -estradiol. The estrogenic activity of *M. oleifera* extract potentiates the action of 17 β-estradiol on the multiplication of vaginal eosinophil cells; hence the cornification of the epithelial cells of the vagina. Jacob and Morris (1969) showed that cornification of vaginal epithelial cells requires a higher peak in estrogen level. Ljungkvist (1971) associated the effect of estrogen with vaginal opening, cornification, growth and proliferation of the endometrium. These results also support the findings of Keshari *et al.* (1996), who reported that hexanic stem bark extract of *Nigella sativa L.*, administered orally, possesses estrogenic activity in immature rats. A similar finding was observed by Dabhadkar and Zade (2012) on the estrogenic activity of *Plumeria rubra* pods.

Histological observations revealed no pathological lesions in the uterus, adrenal glands of control and treated rats. These results also revealed no toxic or pathological effect of the aqueous extract of *M. oleifera* on the treated ovariectomized rats. The results obtained with *M. oleifera* were consistent with those obtained with another plant. Obuchi et *al.* (2009) showed that garlic has no adverse effects on the reproductive system of rats and could be used in the treatment of diseases associated with pregnancy or during pregnancy.

Microscopic observation of the uterine horns showed an increase in endometrial height and an increase in the proliferation of epithelial cells and uterine glands in the groups treated with estrogen, 300 mg/Kg bw of aqueous extract. of *M. oleifera*, to 17ß-estradiol +150 mg/Kg of body weight and 17ß-estradiol +300 mg/Kg of body weight of aqueous extract of *M. oleifera* compared to the control group. Indeed, uterine cellular changes were regulated by circulating levels of ovarian sex steroids progesterone and estrogen (Lessy, 2003). These proliferations are responses of the uterus to estrogen in mice and rats (Huet-Hudson *et al.*, 1989). Estrogens stimulate the rapid renewal of glandular epithelial cells. In the absence of this hormone, the rate of division of endometrial cells is very low (Pollard and Finn, 1974). Heryanto and Rogers (2002) showed that in the uterus of ovariectomized adult rats, under the influence of estrogen, the proliferation of endometrial cells begins within 24 h. These cells precede the growth of endometrial tissue. Estrogen influences the endometrium with activation of the cellular genome through its receptor in the nucleus of a target cell (Horne and Blithe, 2007). Murray (1992) showed that the uterine epithelial cells of ovariectomized sheep undergo morphological alternations thanks to protein-synthesizing organelles.

The increase in the fresh and dry weight of the uterine horns at the dose of 300 mg/Kg bw would indicate that the *M. oleifera* extract appears to contain estrogen agonists. These results were similar to pharmaceutical findings for evaluating the activity of estrogen agonists and their potential therapeutic effect (OECD, 2008). The increase in uterine weight at the dose combinations of 17ß-estradiol + 150 mg/Kg of body weight and 17ß-estradiol + 300 mg/Kg of body weight could therefore be explained by the potentiation of estrogen by the extract of M. oleifera. Indeed, the most frequently measured uterine response is the change in the weight of fresh uterine tissue. Thus, the observed uterine weight rather corresponds to a variety of processes, including improvements in vascularization, permeability, fluid retention, size and elevated number of epithelial cells due to their proliferation (Reel *et al.*, 1996). These processes could therefore explain the high number of uterine glands during the combinations of 17ß-estradiol +150 mg/Kg bw and 17ß-estradiol +300 mg/Kg bw. The extract of *M. oleifera* therefore seems to potentiate the effect of 17ß-estradiol. Similar results were obtained with ovariectomized immature rats. To these rats, the ethanolic extract of *Plumeria rubra* was administered orally between the 11th and 15th days of ovariotomy. This administration resulted in increased uterine weight and stimulation of uterine growth, suggesting estrogenic activity (Dinesh *et al.*, 2012). Another plant, *Derris brevipes* exhibited estrogenic activity. It would act on the significant increase in the weight of the uterine horn, the diameter of the uterus, the thickness of the endometrium and the height of the epithelium of the endometrium and of the vaginal cornification in these immature rats by relationship to the witness (Psychoyos and Prapas, 1987). In ovariectomized mice, studies have shown that treatment with 17β-estradiol alone stimulated the proliferation of uterine epithelial cells (Terada *et al.*, 1989). This observation was also made by Heryanto and Rogers (2002), using an ovariectomized mouse model. They showed that the greatest amount of endothelial cell proliferation was observed in animals that were treated with progesterone alone. In addition, after the injection of a high concentration (overdose) of estrogen, a decrease in the height of the uterine epithelium and stroma as well as signs of degeneration of uterine tissues were observed (Fatemeh *et al.*, 2008). Progesterone affects the uterine glands, while estrogen causes an increase in the size of the epithelial surface of the endometrium (Fatemeh *et al.*, 2008). In the present study, the increase in the size of epithelial cells would be due to *estrogen-like* substances from *M. oleifera* while the proliferation of endothelial glands would be caused by substances that mimic the effect of progesterone in the extract of *M. oleifera*. These substances bind to estrogen and progesterone receptors in the uterine glands.

The increase in the number of animals presenting a vaginal opening clearly confirms estrogenic activity due to the *M. oleifera* extract in ovariectomized pre-pubertal rats. Indeed, Diel *et al.* (2002) showed that the administration of estrogenic or *estrogen-like* substances to pre-pubertal or ovariectomized rats induced an increase in mass through water imbibition of the uterus, cornification and opening of the orifice (Hiremath and Hanumanthara, 1990). The phytoestrogens in *M. oleifera* extract could be suspected of positively influencing the uterine weight of ovariectomized immature rats. The effects obtained could be explained by the fact that estrogens very strongly stimulate the proliferation of uterine endometrial cells as indicated by Roberts and Sporn (1992); Clarke *et al.* (2003). Several authors including Bleu (2012), Blahi (2017) and Zougrou (2018) obtained the same results with aqueous extracts of the respective plants of *Sarcocephalus latipholius*, *Cnestis ferrugenea* and *Passiflora latiflora*.

Some work has shown that plant steroids, flavonoids and saponins are known to exhibit estrogenic properties (Psychoyos, 1966). Lignans like isoflavones bind to estrogen receptors, they exert a weak estrogenic or antagonistic effect similar to tamoxifen at the target tissues, depending on the site of action, the level of endogenous estrogen and the level of the receptors (Sylvie *et al.*, 2003).

The evaluation of the fresh and dry weight of the adrenal glands after seven days of treatment showed no significant change ($p > 0.05$) compared to the batches treated with distilled water and 17 β-

estradiol. These results suggest that the aqueous extract of *M. oleifera* does not influence the physiology of the adrenal glands. These results were also obtained by Blahi (2017) with the aqueous extract of *Sarcocephalus latipholius* on ovariectomized rats. Kozvun (1996) also noted atrophy and significant histological changes in the adrenal glands following ovariectomy performed on adult rats. Kouakou and Benié (2003), for their part, obtained an increase in the weight of the adrenal glands of 83 and 87% with aqueous extracts of Daldinia concentrica and Psathyrelle efflorescens respectively.

Conclusion

At the end of this study, it appears that the aqueous extract of *M. oleifera* would possess phytoestrogens capable of inducing utero-trophic effects on ovariectomized immature rats. These results would explain the use of this plant in the treatment of infertility in certain regions of India. Considering the results, this plant could be integrated into the recipes of improved traditional medicines against infertility.

References

Aké-Assi L. 2002. Flore de la Côte d'Ivoire 2, catalogue systématique, biogéographique et écologie. *Conservatoire et Jardin Botanique.* Genève (Suisse). 401 p.

Blahi A, Nadia M, Zougrou NE, Lavry GN, Kouakou K. 2017. Effects of the aqueous extract of *Sarcocephalus latifolius* (smith) sheets on the reproduction parameters in male rats. The Pharma Innovation Journal **6**(6), 85-91.

Bleu GM, Kouakou K, Zahoui OS, Touré A, Traoré F. 2012. Oral acute toxicity and estrogenic effects of the extracts of *Passiflora foetida* Linn. (Passifloraceae) leaves in female Wistar albino rats. Annals of Biological Research **3**(9), 4609-4616.

Butera PC. 2010. Œstradiol and the control of food intake. Physiology & Behavior **99**(2), 175-180.

Cekmez F, Canpolat FE, Pirgon O, Çetinkaya M, Aydinoz S, Suleymanoglu S. 2011. Apelin, vaspin, visfatin and adiponectin in large for gestational age infants with insulin resistance. Cytokine **56**, 387-391.

Clarke R, Liu MC, Bouker KB, Gu Z, Lee RY, Zhu Y, Skaar TC, Gomez B, O'brien K, Wang Y, Hilakivi-Clarke LA. 2003. Antioestrogen resistence in breast cancer and the role of estrogen receptor signaling. *Oncogene* **22**(47), 7316-7339.

Dabhadkar DK, Zade VS, Rohankar PH, Pare SR, Wikhe MA. 2012. Estrogenic and anti-estrogenic potentials of ethanolic pod extract of *Plumeria rubra* in female albino rats. Global Journal of Pharmacology **6**, 142-147.

Diel P, Schmidt S, Vollmer G. 2002. *In vivo* test systems for the quantitative and qualitative analysis of the biological activity of phytoestrogens. Journal of Chromatography B. **777**, 191-202.

Dinesh KD, Varsha SZ, Pratibha HR, Shital RP, Manisha AW. 2012. Estrogenic and anti-estrogenic potentials of ethanolic pod extract of *Plumeria rubra* in female albino rats. *Global* Journal of Pharmacology **6** (2), 142-147.

Dorfman RI, Gallagher TF, Koch FC. 1936. The nature of the estrogenic substance in human male urine and bull testis. Endocrinology **19**, 33 - 41.

Fatemeh PS, Mojdeh S, Mehdi FM, Mojtaba RV, Ebrahim H. 2008. The changes in morphology and morphometrical indices of endometrium of ovariectomized mice in response to exogenous ovarian hormones. Iranian Journal of Reproductive Medicine **6**, 125-131.

Heryanto B. et Rogers P. A. 2002. Regulation of endometrial cell proliferation by oestrogen and progesterone in the ovariectomized mouse. *Reproduction*. 123: 107-113.

Hiremath SP, Hanumanthara S. 1990. Antifrtility activity of *striga lutea* (Scrophulariaceae) on rats. Contraception **42**, 466-477.

Horne FM, Blithe DL. 2007. Progesterone receptor modulators and the endometrium: changes and consequences. Human Reproduction Update **13**, 567-580.

Hould R. 1984. Techniques d'histopathologie et de cytopathologie. Editor: Centre collégial de développement de matériel didactique. Québec (Province). **399**p.

Huet-Hudson YM, Andrews GK, Dey SK. 1989. Cell typespecific localization of c-myc protein in the mouse uterus: modulation by steroid hormones and analysis of the periimplantation period. Endocrinology **125**, 1683-1690.

Jacob D, Morris JM. 1969. Estrogenic activity of postcoital antifertility compounds. Fertility and Sterility **20,** 211-222.

Jensen EV, Desombre ER. 1972. Mechanism of action of the female sex hormones. Annual Review of Biochemistry 203-230.

Kouakou K, Kati CS, Mangue NEJ. 2008. Effets bénéfiques des extraits d'*Arachis hypogaea* (Fabacée) et de *Phaseolus vulgaris* (Fabacée) sur la perte osseuse chez un modèle animal d'ostéoporose **6**(1), 5-12.

Kouakou K, Benié T. 2003. Effet antifertilisant de *Daldinia concentrica* et *Psathyrella efflorescens*. Recherche des effets oestrogéniques. Ethnopharmacologia **31**, 45-57.

Kouakou KR, Tahiri A, Kouakou K. 2018. Effect of aqueous extract of leaves of *Moringa oleifera* (Moringaceae) LAM. 1785 on the reproductive cycle of animal model *Rattus norvegicus* (Murine) wistar strain. European Journal of Biotechnology and Bioscience **6**(6), 37-42.

Kozvun OI. 1996. The effect of ovariectomy and œstradiol on DNA, RNA and protein metabolism in adrenal cortex of rats. Fiziologichnyi Zhurnal **42**(1), 53-58.

Lessy BA. 2003. Two pathways of progesterone action in the human endometrium: implications for implantation and contraception. Steroids **68**, 809-815.

Ljungkvist I. 1971. Attachment reaction of rat uterine luminal epithelium. The effect of œstradiol estrone and estriol on the morphology of the luminal epithelium of spayed virgin rats. Acta of the Society of Medicine Uppsala **76**, 139- 157.

Ljungkvist I. 1971. Attachment reaction of rat uterine luminal epithelium. The effect of œstradiol estrone and estriol on the morphology of the luminal epithelium of spayed virgin rats. Acta of the Society of Medicine Uppsala **76**, 139- 157.

Murray MK. 1992. The effect of estrogen and progesterone on structural changes in the uterine glandular epithelium of the ovariectomized sheep. Biology of Reproduction **47**, 408-417.

Obuchi GO, Malu SP, Obi-Abang M, Alozie Y, Iyam MA. 2009. Effect of garlic extracts on Monosodium Glutamate (MSG) Induced fibroid in Wister rats. Science Alert **8**(7), 970-976.

OCDE. 2008. Bioessai utérotrophique chez les rongeurs: un test de dépistage à court terme pour les propriétés œstrogéniques. 21p.

Panda S, Kar A, Sharma P, Sharma A. 2013. Cardioprotective potential of N,α-Lrhamnopyranosyl vincosamide, an indole alkaloid, isolated from the leaves of Moringa oleifera in isoproterenol induced cardiotoxic rats: *In vivo* and in vitro studies. Bioorganic and Medicinal Chemistry Letters **23**(4), 459-462.

Philippi C. 1997. *Moringa* l'arbre aux usages Multiples. Thèse de Docteur d'Etat en Pharmacie. Université Henri Poincaré - Nancy I. **178**p.

Pollard RM, Finn CA. 1974. The effect of ovariectomy of puberty on cell proliferation and differentiation in the endometrium of the aged mouse. Biology of Reproduction **10**, 74-77.

Psychoyos A. 1966. Recent research on egg implantation, CIBA Foundation Study Group. **23** p.

Psychoyos A, Prapas I. 1987. Inhibition of egg development and implantation in rats after postcoital administration of the progestrerone antagonist RU 486. Journal of Reproduction and Fertility **80**, 487-491.

Purwal L, Pathak AK, Jain UK. 2010. *In vivo* anticancer activity of the leaves and fruits of *Moringa oleifera* on mouse melanoma. *Pharmacologyonline* **1**, 655-665.

Reel JR, Lamb JC, Neal BH. 1996. Survey and assessment of mammalian estrogen biological assays for hazard characterization. Fundamental and Applied Toxicology **34**, 288-305.

Roberts AB, Sporn MB. 1992. Mechanistic interrelationships between two superfamilies: the steroid/retinod receptors and transforming growth factor-b. Cancer Surveys **14**, 205-220.

Saengsirisuwan V, Pongseeda S, Prasannarong M, Vichaiwong K, Toskulkao C. 2009. Modulation of insulin resistance in ovariectomized rats by endurance exercise training and estrogen replacement. Metabolism **58**(1), 38-47.

Shaila G, Mahjan, Anita A. 2010. Immunosuppresive activity of ethanolic extract of seeds of *Moringa oleifera* Lam in experimental immune inflammation. *Ethnopharmacol Commun* **130**, 183–186.

Sripriya S, Yuvaraj G, Nema RK, Kumar VM, Deecaraman M. 2011. Evaluation of Antifertility activity from Stem Part of *Ocimum gratissimum* in Acetone extracts. International Journal of Pharmaceutical and Clinical Research **3**(2), 41-44.

Sylvie D, Claudine B, Isabelle M. 2003. Phytoestrogènes chez la femme ménopausée. Medecine/Sciences **19**, 1030-1038.

Terada N, Yamamoto R, Takada T, Miyake T, Terakawa N, Wakimoto H, Taniguchi H. 1989. Inhibitory effects of progesterone on cell death of mouse uterine epithelium. Journal of Steroid Biochemistry **33**, 1091-1096.

T**ouré D.** 2015. Etudes chimique et biologique des huiles essentielles de quatre plantes aromatiques medicinales de côte d'ivoire. Thèse de Doctorat en Biologie Humaine Tropicale. Université Félix Houphouët- Boigny Abidjan. Archives-ouvertes, **116**p.

Yang R, Lien-Chung C, Jenn-Chung H, Weng BC, Palada CM, Chadha ML, Levasseur V. 2006. Nutritional and functional properties of *Moringa* leaves from germplasm, to plant, to food, to health. Strategies, standards and markets for a better impact on nutrition in Africa. Accra, (Ghana). 16-18.

Zirihi GN, Kra AM, Guédé-Guina F. 2003. Évaluation de l'activité antifongique de *Microglossa pyrifolia* (LAMARCK) O. KUNZE (ASTERACEAE) << PYMI>> sur la croissance in vitro de *Candida albicans*. *Revue de méecines et pharmacopés africaines* **17** 11-18.

Zoth N, Weigt C, Laudenbach-Leschowski U, Diel P. 2010. Physical activity and estrogen treatment reduce visceral body fat and serumlevels of leptin in an additive manner in a diet induced animal model of obesity. Journal of Steroid Biochemistry and Molecular Biology **122**, 100-105.

Zougrou NE, Blahi AN, Kouassi KD, Kouakou K. 2018. Effects of the Aqueous Extract of *Cnestis Ferruginea* on the Histological Structure of Female Rat Ovary and Uterine Horns. Research. Biomed J Sciences & Technical. **2**(1), 1-6.