

Effects of *Passiflora foetida* Linn. (Passifloraceae) on Genital Tract, Serum Estradiol, Pituitary Gonadotropin and Prolactin Level in Female Adult and Immature Ovariectomized Rats

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Abstract. The effects of the extracts of *Passiflora foetida* on the genital tract, serum estradiol, pituitary gonadotropin (LH and FSH) and prolactin were studied in female adult (120-140 g) and immature ovariectomized (30-40 g) rats. Results showed that the aqueous extract increased significantly both the ovary and uterus weight whereas the hexane extract increased the weight of the ovary only and the methanol extract increased the weight of uterus only. Histological examination of these organs indicated that *P. foetida* treated rats were in estrous or proestrous phases of the estrous cycle. The hormone analysis showed that the serum LH was significantly increased by 17 β estradiol and by the three extracts dose dependently in immature ovariectomized rats. Moreover, the aqueous extract increased significantly serum estradiol and pituitary gonadotropins and prolactin in adult non ovariectomized rats.

Keywords: *Passiflora foetida*, ovary, uterus, estradiol, gonadotropins, prolactin

Introduction

According to the World Health Organization, 80% of the population in Africa depends on traditional medicine for their health-care needs (WHO, 2002). This continent is endowed with a rich biodiversity estimated about 40,000 plant species (Mahunnah, 2002) and more than 4000 species are used medicinally (Bosh *et al.*, 2002). Infertility is one of the most crucial health problems which are treated in the African traditional medicine. This reproductive problem causes divorces, instability and polygamy in many families where some women considered as sterile are victims of violence (Papreen *et al.*, 2000; Unissa, 1999). Hence, most of them use medicinal plants to recover their fecundity (Kambu-Kabangu, 1990) due to poverty. *Passiflora foetida* is a medicinal plant used by population in the south of Côte d'Ivoire to cure infertility in women (N'Guessan, 2008; 1995). Furthermore, it serves to treat other diseases such as snakebites, epilepsy, and abscess (N'Guessan, 2008; 1995; Adjanohoun and Aké-Assi, 1979). It is used in Asia continent as emmenagogue and as remedy for asthma, biliousness, hysteria, giddiness and headache (Dhawan *et al.*, 2004). The major phytochemical consti-

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tents of this plant are flavonoids, glycosides, phenolic compounds, fats, steroids and alkaloids (Patel *et al.*, 2011; Ingale and Hivrale, 2010; Echeverri *et al.*, 2001; Cambie and Ash, 1994). This study was aimed to evaluate the effects of *Passiflora foetida* leaves on the genital tract, estrogen and pituitary hormone levels in female wistar rats.

Materials and Methods

Plant material. The leaves of *P. foetida* were collected from the north and the south of Abidjan, the economic capital city of Côte d'Ivoire and authenticated in the Laboratory of Botany (UFR Biosciences) of the University of Cocody, Abidjan. A voucher specimen was deposited in the botanical garden of this University under the number 746B.

Preparation of the extracts. The collected plant material was dried at an ambient temperature (30 \pm 2 °C) without exposure to sun light and crushed to obtain a powder which was divided into three parts, 50 g of each were macerated separately for 24 h in water (1500 mL), hexane (750 mL) and methanol 95° (750 mL), filtered using Whatman filter paper number 1 and concentrated in an air circulating oven at 50 °C until total dryness.

The aqueous, hexane and methanol extracts obtained (yield 20.23%, 6.83% and 28.03% respectively) were then stored at 4 °C in a refrigerator for the experimental studies.

Effects on the genital tract. Adult female wistar albino rats (120-140 g) were divided into groups of 5 rats each and administered separately the dose of 500 mg/kg body weight of each extract for 14 and 28 days. The control group was treated with olive oil. All the treatments were given orally, using an intragastric cannula. At the day after the last treatment, the rats were weighed and sacrificed by decapitation under light ether anesthesia. The ovary and uterus of each rat were dissected out and weighed rapidly using a sensitive balance. The adrenal and kidney were also dissected and weighed. Additionally, the dissected organs were fixed in bouin's fluid for 24 h, dehydrated and embedded in paraffin. The paraffin sections were cut at 4 µm and stained with haematoxylin-eosin for histological examinations.

Effects on LH level of immature ovariectomized rats. The immature female albino rats (30-40 g) were bilaterally ovariectomized (ovx) under light ether anesthesia through lateral incision in the skin just below the last rib (Keshri *et al.*, 1995; Zarrow *et al.*, 1964). After a post operative rest period of 7 days, the rats were divided into 8 groups of 6 animals each and treated separately with olive oil (control group), 0.02 mg/kg body weight of 17β estradiol, the high dose (500 mg/kg body weight) and low dose (250 mg/kg body weight) of each extract. All the treatments were given orally for 7 consecutive days and 24 h after the last treatment, the rats were anesthetized with ether and sacrificed by

decapitation. The blood samples were collected into non heparinized tubes and centrifuged at 2580 rpm for 10 min. The serum was then separated and stored at -20 °C until LH analysis by the ELFA technique (Enzyme Linked Fluorescent Assay) using specific kit (BioMerieux, Lyon, France).

Effect on serum estrogen, gonadotropins and prolactin of adult rat. The aqueous extract was found to be the most active extract on the genital tract and on LH level of ovx rat. Hence, it was used to determine the effect of *P. foetida* on estrogen and pituitary hormones. For this experiment, female adult rats were divided into two groups of 5 rats each. The first group served as control and received olive oil only and the second group was treated with a dose of 500 mg/kg body weight of the aqueous extract by gavage for 28 consecutive days. At the end of the treatment, the rats were sacrificed under ether anesthesia and the collected blood samples were analyzed for the level of estrogen (E₂), gonadotropin (LH and FSH) and prolactin (PRL) in the serum by the same methods used in the previous experiment.

Statistical analysis. The data were expressed as mean±SEM. Statistical analysis of the variance between control and experimental values were done using student's t-test. A probability level of less than 5% (p<0.05) was considered significant.

Results and Discussion

Effects on ovary and uterus weight. The effect of *P. foetida* on the weight of ovary, uterus, adrenal and kidney of adult female rats are presented in the Table 1. The extracts did not induce any change in the weight

Table 1. Effects of *P. foetida* on the weight of ovary, uterus, adrenal and kidney of female adult rats

Treatments	Duration (days)	Organ weight (mg/100 g body weight)			
		Kidney	Ovary	Uterus	Adrenal
Control (olive oil)	14	274.79±21.88	16.80±3.69	152.63±12.67	13.65±1.47
	28	276.41±20.86	17.50±4.44	151.45±14.85	13.81±2.32
AE (500 mg/kg)	14	265.43±19.65	28.65±3.77**	188.19±18.06**	13.22±2.18
	28	268.32±13.84	25.27±3.21*	216.89±35.07**	12.83±2.39
HE (500 mg/kg)	14	262.42±12.73	27.74±7.23*	133.98±35.83	12.76±3.29
	28	273.39±26.87	28.82±3.49***	143.79±37.54	11.48±1.76
ME (500 mg/kg)	14	295.91±23.22	17.67±2.90	189.04±13.94**	12.37±2.71
	28	283.77±8.94	16.65±3.72	225.44±31.29**	13.26±1.31

Values are means±SEM (n=5); * = p<0.05; ** = p<0.01; *** = p<0.001; AE = aqueous extract; HE = hexane extract; ME = methanol extract.

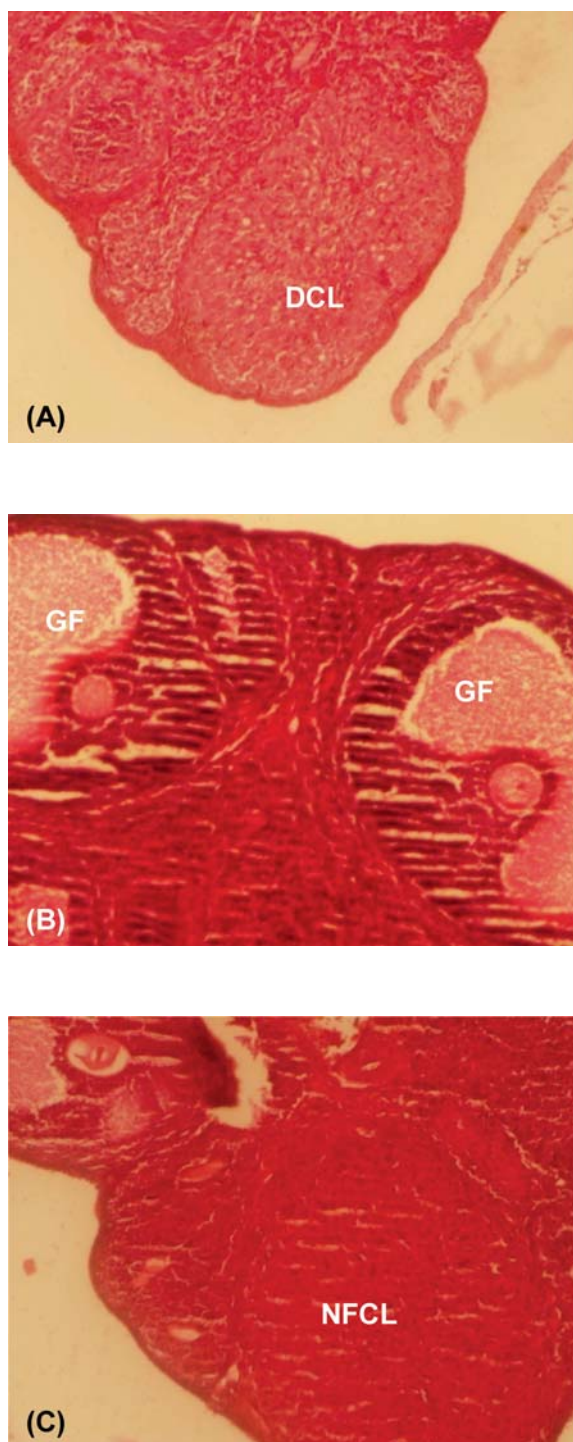


Fig. 1(A-C). Photomicrograph of the ovaries in rats showing degenerated corpora lutea (DCL) in control rats (A) and Graafian follicles (GF) and newly formed corpora lutea (NFCL) in *P. foetida* treated rats (B and C). Original magnification $\times 10$; stained: Haematoxylin-eosin.

and histological structure of kidney and adrenal. However, the aqueous extract induced significant increase in the weight of ovary and uterus after 14 days and 28 days of treatment. The hexane extract induced only significant increase of ovary weight whereas the methanol extract increased significantly only the uterine weight after 14 and 28 days of treatment when compared to control group. Furthermore, the 14 days treatment-induced weight changes were not significantly different from those of the 28 days treatment.

The histological examination of the ovaries of both the aqueous and hexane extracts treated groups showed the presence of newly formed corpora lutea or Graafian follicles in all the rats. Control group showed degenerated corpora lutea without any presence of Graafian follicles in 80% of the rats (Fig. 1A-C). Histological evaluation of the uterus indicated that the endometrial epithelium of the aqueous and methanol extract-treated rats consisted of tall columnar cells with apoptotic and/or mitotic figures. Endometrial epithelium of the control rats included small, low columnar cells (Fig. 2A-B).

Effects on serum level of LH of ovx rats. There was a dose dependent increase in LH in the serum of immature ovx rats by all the extracts and by 17β estradiol. However, the effect of the extracts at the dose of 250 mg/kg was greater than that induced by the dose of 500 mg/kg. The aqueous extract was found to be the most effective extract at the high dose (500 mg/kg) whereas the methanol extract was the most effective one at the low dose (250 mg/kg) (Fig. 3).

Effect on E_2 and the pituitary hormones of adult rats. The aqueous extract of *P. foetida* caused significant increase of gonadotropins (LH and FSH) and prolactin level in the serum of the adult female rats (Fig. 4A-B). The serum estrogen of the rats was also highly increased (Fig. 5).

The extracts did not change the weight or the histological architecture of the adrenal and kidney. So, *P. foetida* had no effect on these organs at doses up to and including 500 mg/kg. However, the aqueous and hexane extracts caused significant increase in the ovary weight. These results are similar to those obtained by Thakur *et al.* (2009) with the ethanolic and aqueous extracts of *Carum carvi* and *Curcuma longa* and with ethinyl estradiol. They are also comparable to the effect of the phytoestrogen resveratrol on ovary weight (Henri and Witt, 2002). Estrogen is a steroid hormone synthesized by the ovary which regulates the physiology of the

female genital tract (Freeman, 2008; Wassarman and Albertini, 1994). It can induce the increase of the ovarian weight by stimulating development and maturation of follicles (Wassarman and Albertini, 1994; Vaissaire, 1977; Smith and Bradbury, 1961). It is suspected that effects of the aqueous and hexane extract are the result of inherent phytoestrogens in the plant. The histological analysis of ovaries showed the presence of newly formed corpora lutea or Graafian follicles in all the treated rats indicating that these rats were in estrous or in proestrous phases of the estrous cycle, respectively. Control group

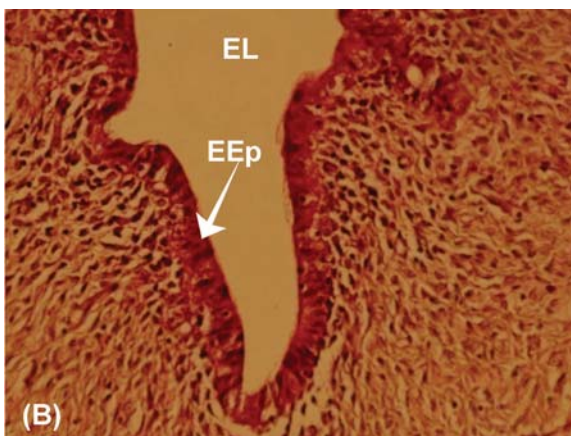
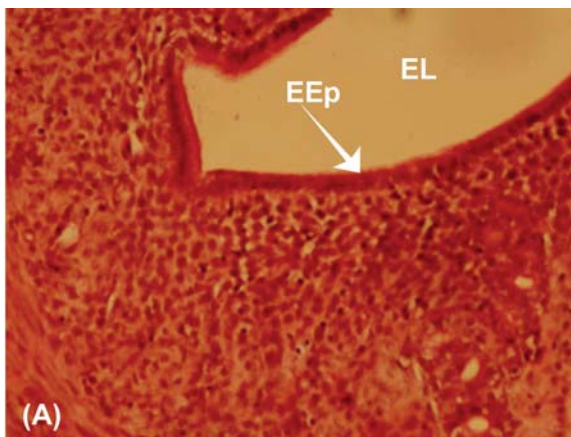


Fig. 2(A-B). Photomicrograph of the uterus showing low, small columnar endometrial epithelium cells in control rats (A) and tall columnar endometrial epithelium cells in the *P. foetida* treated rats (B). E = Endometrium; EEp = Endometrial epithelium; EL = Endometrial lumen. Original magnification $\times 10$; stained: Haematoxylin-eosin.

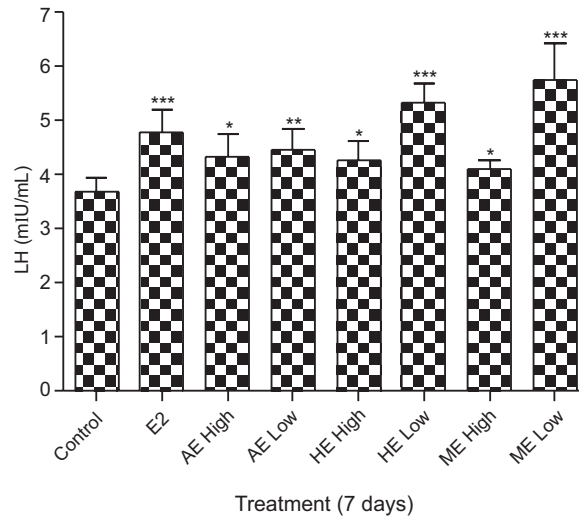


Fig. 3. Effects of 17β estradiol and *P. foetida* on serum LH of immature OVX rats. Values are means \pm SEM (n=6); *p < 0.05; **p < 0.01; ***p < 0.001.

rats showed degenerated corpora lutea without presence of Graafian follicles comparable to those of metestrous and diestrous phases. Estrogen is well known to stimulate gonadotropins surge which induce follicles development and ovulation (Freeman, 2008; Haim *et al.*, 2003; Mora *et al.*, 1994). This result confirmed the potential estrogenic effect of these extracts. The uterine wet weight in this experiment was also increased by the treatment with the aqueous and methanol extracts. The same results have been obtained by Thakur *et al.* (2009) and Kouakou (2000) after administration of 17β estradiol to adult rats. Estrogen stimulates uterus weight, endometrial proliferation and hyperplasia by production of insulin-like growth factor I (IGF-I), expression of progesterone receptor and the complement protein C3 (Albertazzi and Sharma, 2005; Seidlova-Wuttke *et al.*, 2003). The fact that *P. foetida* aqueous and methanol extracts increased the uterine weight suggests that they acted as estrogen-like, mimicking probably the estrogen mechanism of action in this organ. The histological examination of the uteri corroborated the weight changes since the endometrial epithelium of *P. foetida* treated group was composed of tall columnar cells with apoptotic and/or mitotic figures. The endometrium of the control rats was not proliferated and the epithelium consisted of small, low columnar cells.

As well as the genital tract, the hypothalamo-pituitary complex is a target organ of estrogen where it influences

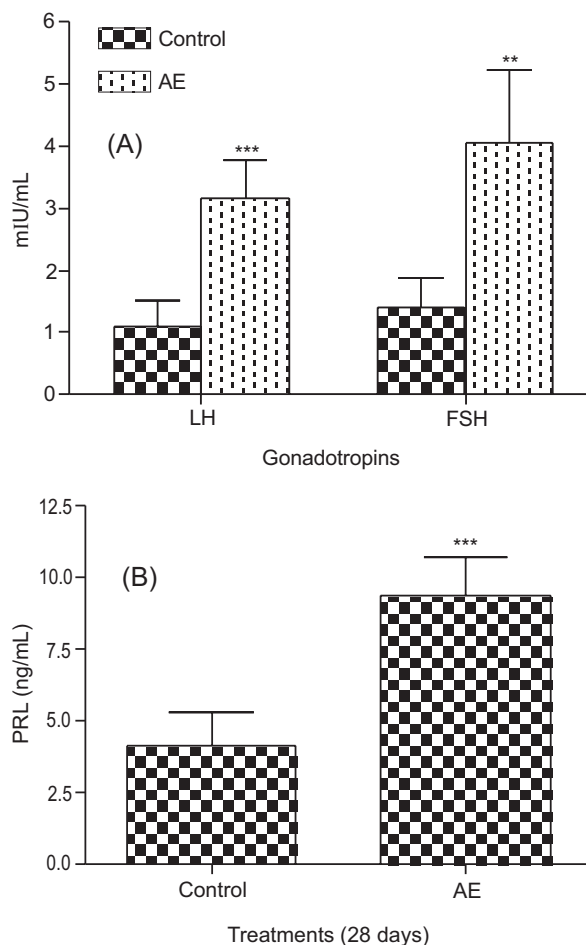


Fig. 4(A-B). Effects of *P. foetida* on pituitary gonadotropins (A) and prolactin (B) level in serum of adult female rats. Values are means \pm SEM(n=5), **p<0.01; ***p<0.001.

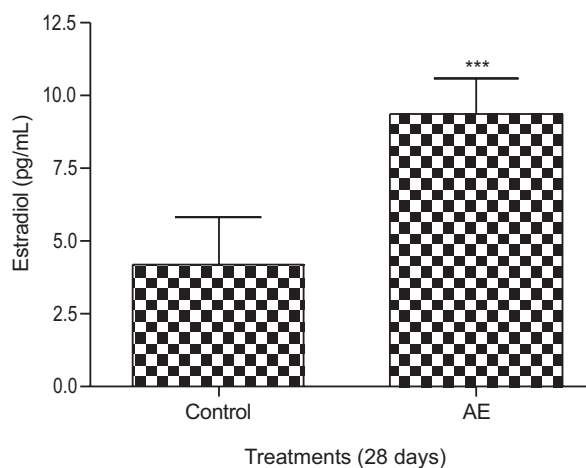


Fig. 5. Effects of *P. foetida* on serum estradiol of adult female rats. Values are means \pm SEM (n=5), ***p<0.001.

indirectly the reproductive cycle using his receptor ER α and ER β (Hraboyszky *et al.*, 2000; Laflamme *et al.*, 1998; Shughrue *et al.*, 1997). In this study, when administered to immature ovx rats, 17 β estradiol and the extracts of *P. foetida* increased significantly the LH level in serum. Several earlier authors had reported similar results (Shinnosuke and Masahico, 1994; Shupnik et Rosenzweig, 1991; Rosen *et al.*, 1989). Folliculogenesis and the secretion of estrogen in the ovary depend on gonadotropin (Monniaux *et al.*, 2009; Wassarman and Albertini, 1994; Hodgen, 1989) but estrogen can modulate their secretion by feed back control (Christoffel *et al.*, 2006; Shupnik, 1996; Garib *et al.*, 1990). It is assumed that increase of LH in serum of ovx rats is the result of phytoestrogens present in the plant. The estrogenic nature of *P. foetida* was confirmed by the aqueous extract tested in adult non ovx rats to determine the effects on the pituitary hormones (LH, FSH and PRL) and on estrogen. All these hormones were significantly increased by the treatment, showing that the estrogenic molecules in this plant did not compete with the endogenous estrogen, acting as agonists. The effects of *P. foetida* may be due to its phytochemical constituents such as flavonoids, alkaloids, steroids, polyphenols (Patel *et al.*, 2011; Ingale and Hivrare, 2010; Cambie and Ash, 1994), substances which are known for their estrogenic effects (Diel *et al.*, 2004; Nazrullaev *et al.*, 2001; Baker *et al.*, 1999). Hence, The results in this study were different from those obtained with the antifertility plants *Trichosanthes cucumerina* var *cucumerina* (Kage *et al.*, 2009), *Afromosia laxiflora*, *Pterocarpus erinaceus* and *Cola nitida* (Benie *et al.*, 2003) which were found to have antiestrogenic effects by reducing ovary weight and serum level of gonadotropin in adult non ovx rats.

Conclusion

P. foetida extracts exerts estrogen-like effects on female genital tract and pituitary gland. Further investigations must be carried out to isolate the active constituents and to elucidate their mechanism of action for the efficient use of this plant in the human reproductive problems.

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