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Fertility enhancing effects of aqueous extract of leaves of *Cnestis ferruginea* Vahl ex De Cantolle on female wistar rats

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Abstract

Cnestis ferruginea (Connaraceae) is one of the plants used as a therapeutic agent in many cultures in tropical Africa. To evaluate the pharmacological effects of the aqueous extract of *C. ferruginea* on reproductive parameters of female rats. Selected regular cycle female rats were randomized into 2 sets of 18 each and treated for 15 (set I) and 30 days (set II). Each set was then divided equally into three groups. Group 1 (control) was orally administered with distilled water once a day. Group 2 and 3 were respectively treated with 50 and 100 mg/kg body weight. Estrous cycle pattern was monitored before and during plant extract application whereas reproductive organs and reproductive hormones were determined at the end of each treatment. *C. ferruginea* induced a blockage of the estrous cycle at the estrous phase. Thus, animals treated showed highly significant increase ($p<0.001$) in the duration of estrous phase. $AECF_{50}$ induced significant increase in the wet weight of ovary (42.690±4.21), $p<0.05$ and (44.470±922), $p<0.001$ and uterus (79.030±8.07), $p<0.05$ and (80.320±1.140), $p<0.001$ after 15 and 30 days of treatment respectively. Whereas, $AECF_{100}$, induce only a significant increase (39.000±1.588, $p<0.01$) in the wet weight of ovary after 30 days treatment. For the dry weight, only $AECF_{50}$ induced a significant increase (0.032±0.002, $p<0.05$) in the weight of uterus. For both duration of treatment and both treatment group, extract produced significant increase in serum concentration of FHS ($p<0.001$), LH ($p<0.01$), estradiol ($p<0.01$). Extract produced significant increase ($p<0.01$) in serum concentration of prolactin after 30 days treatment. The present study suggests that the extract of *Cnestis ferruginea* could contain estrogenic compounds favourable to fertility optimization.

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Introduction

Socio-cultural and economic reasons make traditional medicine first medication choice in developing countries. The essential components of this medicine in Africa are plants (Sandu and Heinrich, 2005; Gupta *et al.*, 2005). Among these medicinal plants in West Africa there is *Cnestis ferruginea* Vahl Ex De Cantolle (Connaraceae).

This plant has a wide distribution in West Africa and generally in tropical Africa (Burkill, 1985). In Côte d' Ivoire and Senegal, it is used in the treatment of ocular disorders (Kerharo 1974; Kerharo and Adam, 1974; Okafor and Ham, 1999). This plant is also used in reproduction as emmenagogue, abortifacient and aphrodisiac (Gill, 1992; Okafor and Ham, 1999; N'guessan *et al.*, 2006).

Pharmacological studies on *C. ferruginea* showed that this plant possesses bioactive substances necessary for bacterial inhibition (Boakye Yiadom and Konning 1975; Akharaiyi *et al.*, 2012). It has been also demonstrated anticonvulsive (Declume *et al.*, 1984), antioxidant (Boakye-Yiadom and Konning 1975; Akharaiyi *et al.*, 2012) and antistress (Ishola *et al*, 2007; Yakubu *et al.*, 2011) activities of this plant extract.

In the field of the reproduction, the aqueous extract of the roots of *C ferruginea* at the doses of 13; 26 and 52 mg/kg of body weight restores sexual competence (Yakubu and Nurudeen, 2012). *Cnestis ferruginea* (Vahl ex De Cantolle) is used by the populations for various other purposes. Indeed, the beautiful red fruits of this plant make of it a decorative plant (Burkill, 1985). It has also nutritional values (Irvine, 1961).

Many chemical compounds have been isolated from different parts of *C. ferruginea*. Thus, the petroleum ether fraction of the fruit showed that it contains components such as: octacosanyl stearate and 1-myristo-2-stearo-3-palmitin (Ogbechie *et al.*, 1987). Parvez and Rahman (1992) have demonstrated the presence of isoflavone glycoside, afrormosin-7-O-beta-D-galactoside in the testa of fruit.

The aqueous extract of the roots of *C. ferruginea* contains alkaloids (24.6 mg/L), flavonoids (14.6 mg/L), saponin (4.6 mg/L), anthroquinines (0.3 mg/L) and tannins (0.1 mg/L) (Yakubu *et al.*, 2011).

The use of this plant as reproductive health care alternative and extension need scientific studies using modern techniques of investigation. Hence, the objective of this study is to evaluate the pharmacological effects of the aqueous extract of *C. ferruginea* on reproductive parameters of female rats

Materials and methods

Plant material

Fresh leaves of *Cnestis ferruginea* were harvested in November in the Region of Nawa, Department of Soubré, precisely in the village named Trawininkro (V8) (Côte d'Ivoire). A sample of this plant has been identified and authenticated by Professor Aké-Assi at the Laboratory of Botany and Plant Biology of Université Félix Houphouët-Boigny.

Preparation of extract

Harvested leaves have been rinsed with distilled water, dried in the shade (sheltered from the sun) at an ambient temperature (30±2 °C). The dried leaves were crushed with a power mill (Retsch SM 100, Germany) to obtain a powder.

The powder obtained has been macerated by mixing 50 g and 1.5 L of distilled water and stirred for 24 hours by a magnetic stirrer (Janke & Kuntelika, Germany). After three times filtration on Whatman filter paper number 1, the filtrate was concentrated in an air circulating oven at 50 °C until total dryness. The aqueous extract obtained (yield 11.51%) has been stored at 4 °C in a refrigerator for the experimental studies.

Animal material

Adult female rats, (*Rattus norvegicus*, Muridae), Wistar strain, virgin, weighing between 130-150 g and aged 55-65 days are from the animal facility of the Faculty of Pharmaceutical and Biological Sciences.
These rats have been used for pharmacological studies of the aqueous extract of *C. ferruginea*.

They were raised in stable temperature room (24±2°C). In these premises, the photoperiod was 12 hours and 50-55% humidity. The animals were fed *add libitum* water, bread, fish, corn and peanuts.

Experimental design

Estrus cycle monitoring

Pre-treatment phase

Animals were acclimatized in the laboratory for two weeks. Then the estrous cycles were monitored for a 20 days. Thus, vaginal smears were examined every morning between 8:00 and 10:00 a.m. Smears were prepared as described by Sahar *et al.* (2007). The staining technique of Haris-Shorr was used to stain the smears. The female rats that have undergone four successive 4 days cycle were selected for this study.

Treatment phase

Thirty-six adult female rats with regular estrous cycle (4 days) were randomly distributed into 2 sets of 18 animals each and treated for 15 (set I) and 30 days (set II). Each set was then divided equally into three groups and treated as follows:

Group 1 (control) was orally administered with distilled water once a day. Group 2 and group 3 were respectively treated with 50 and 100 mg/kg body weight of aqueous extract of *C. ferruginea* orally once a day. Vaginal smear were monitored like previously.

Body weight and organ weight

The body weight of each animal was recorded all the two days during treatment. After 24 hours of last treatment, the final weight was recorded and the animals were sacrificed by cervical dislocation.

Uterine horns length and organ weight

Immediately after the sacrifice of animals, the abdominal cavity is opened and the uterine horns are measured *in situ*. The ovary, uterine horn, cervix and adrenal gland of each rat were dissected out, weighed quickly using a sensitive balance (wet weight). These weighed organs, except ovary are placed at the drying oven at 100°C during 24 hours and weighed again (dry weight).

Reproductive hormone levels

During the sacrifice, blood was collected. Sera were separated by centrifugation 3000 r/min for 10 minutes and stored at -20°C until used for the assessment of FSH, LH, estradiol, progesterone and prolactin levels by the ELFA technique (Enzyme Linked Fluorescent Assay) using specific kits (Bio Merieux, Lyon, France).

Statistical analysis

The data and graphical representation of the data was performed using the Graph Pad Prism 5.01 software (Microsoft, USA). The experimental results were expressed as Mean ± SEM and data were assessed by the method of analysis of one-way ANOVA followed by Tukey test with least significant test. P value <0.05 was considered significant, P value <0.01 considered highly significant and P value <0.001 considered very highly significant.

Results

Effects of *C. ferrugenea* on rat body weight

The increase in body weight of the treated rats did not show any significant difference (p> 0.05) compared to control (Figure 1). At the end of treatment, the increase in weight of control rats was 13.00±0.82% compared to their initial weight. Those of treated rats had increased of 14.89±3.16% and 15.29±1.47 respectively for doses of 50 mg/kg B.W ($AECF_{50}$) and 100 mg/kg B.W ($AECF_{100}$)

Effects of C. ferruginea leave extract on the rat estrous cycle

The aqueous extract of *C. ferruginea* induced a disruption followed by the blockage of the estrous cycle at the estrous phase after 15 and 30 days of treatment with both doses ($AECF_{50}$ and $AECF_{100}$) as shown in Table 1.

Effects of C. ferruginea leave extract on the evolution of vaginal cells of the treated animals

Figure 2 represents the curves expressing the daily evolution of proportion of the cornified cells and leukocytes obtained during the various vaginal smears.

Thus, A represents the case of a control rat and B and C the case of treated rats ($AECF_{50}$ and $AECF_{100}$).

Effects of C. ferruginea extract on the duration of different phases in estrus cycle

With regard to each estrous cycle stage, animals treated for 15 days and 30 days showed highly significant increase ($p < 0.001$) in the duration of estrous phase for the two doses ($AECF_{50}$ and $AECF_{100}$). Metestrus and diestrous phase in both doses and both duration of plant extract treated-rat, were highly significantly decreased ($p<0.001$).

The duration of diestrous of treated-rat, was highly significantly decreased ($p<0.001$) for 30 days treatment and the both doses.

Table 1. Effect of aqueous extract of *C. ferruginea* on the estrus cycle.

Treatment groups	Number of rat	Percentage of rats blocked in estrus					
		5th day	10th day	15th day	20th day	25th day	30th day
Control	6	0	0	0	0	0	0
$AECF_{50}$	6	33.33	66.66	50	83.33	100	100
$AECF_{100}$	6	33.33	66.66	100	100	100	100

Control: Distilled water

$AECF_{50}$: Aqueous Extract of *C. freruginea* (50 mg/kg of body weight)

$AECF_{100}$: Aqueous Extract of *C. freruginea* (100 mg/kg of body weight).

The duration of 15 days treated-rat showed a highly significant decrease ($p < 0.001$) of duration of diestrous phase for the dose of 100 mg/kg of body weight. Whereas $AECF_{50}$ significantly decreased ($p<0.01$) the duration of diestrous phase. Concerning the proestrous, the light increase in the duration of all animals treated with the plant extract is not significant (Figure 3).

Effects of C. ferruginea on ovary, uterine horn, cervix and adrenal gland relative wet and dry weights

The effect of *C. ferruginea* after 15 days of treatment on the wet weight of ovary, uterus, cervix and adrenal and the dry weight of uterus, cervix and adrenal of adult female rats are presented in the Table 2.

Table 2. Effect of C. ferrugina on the wet and dry weight of reproduction organs and adrenal gland of female adult rats after 15 days treatment.

Treatment groups	Wet weight (mg/100g b.w.)				Dry weight (mg/100g b.w.)		
	Ovary	Uterus	Cervix	Adrenal	Uterus	Cervix	Adrenal
Control	25.800±2.89	50.780±5.16	43.470±1.545	15.840±2.05	0.020±0.002	0.013±0.001	0.005±0.000
$AECF_{50}$	42.690±4.21*	79.030±8.07*	46.020±4.100	12.410±1.39	0.020±0.003	0.014±0.002	0.005±0.000
$AECF_{100}$	27.170±1.99	61.280±2.43	42.730±2.136	11.090±1.28	0.016±0.001	0.012±0.002	0.004±0.000

Values are means ± SEM (n=6); * = $p<0.05$. Control: Distilled water, $AECF_{50}$: Aqueous Extract of *C. freruginea* (50 mg/kg of body weight), $AECF_{100}$: Aqueous Extract of *C. freruginea* (100 mg/kg of body weight).

For both doses ($AECF_{50}$ and $AECF_{100}$), the extract did not induce any change in the wet weight of cervix and adrenal when compared to control group. However, $AECF_{50}$ induced significant increase ($p<0.05$) in the wet weight of ovary and uterus after 15 days of treatment when compared to control group.

With regard to the dry weight of 15 days treatment groups, the extract did not induce any change when compared to control group.

Extract treated rats after 30 days showed a significant increase ($p<0.001$) of uterus and ovary wet weight at the dose of 50 mg/kg of body weight.

Whereas, $AECF_{100}$, induce only a significant increase ($p<0.01$) in the wet weight of ovary when compared to the control group.

For the dry weight, only $AECF_{50}$ induced a significant ($p<0.05$) increase in the weight of uterus when compared to control group (Table 3).

Table 3. Effect of *C. ferrugina* on the wet and dry weight of reproduction organs and adrenal gland of female adult rats after 30 days treatment.

	Wet weight (mg/100g b.w.)				Dry weight (mg/100g b.w.)		
Treatment groups	Ovary	Uterus	Cervix	Adrenal	Uterus	Cervix	Adrenal
Control	26.530±1.735	51.640±3.287	37.440±6.098	12.220±1.708	0.021±0.002	0.011±0.002	0.004±0.000
$AECF_{50}$	44.470±2.922***	80.320±1.140***	41.180±5.133	12.350±0.945	0.032±0.002*	0.011±0.003	0.003±0.000
$AECF_{100}$	39.000±1.588**	53.930±2.677	38.350±2.926	11.890±1.120	0.021±0.003	0.011±0.002	0.003±0.000

Values are means ± SEM (n=6); * = $p<0.05$; ** = $p<0.01$; ***= $p<0.001$. Control: Distilled water, $AECF_{50}$: Aqueous Extract of *C. freruginea* (50 mg/kg of body weight), $AECF_{100}$: Aqueous Extract of *C. freruginea* (100 mg/kg of body weight).

The effect of *C. ferruginea* on the uterine horn length

The effect of leaves aqueous extract of *C. ferruginea* is summarized in Table 4. Indeed, $AECF_{50}$ induced significant increase of the length of both right and left uterine horn after 15 days and 30 days treatment when compared to control group. After 15 days of treatment, $AECF_{100}$ induced significant increase in both right and left uterine horn.

Effects of C. ferrugina on the pituitary and ovary hormones

The effects of administration of aqueous extract of *C. ferrugina* leaves at 50 and 100 mg/kg body weight for 15 and 30 days on the concentration of serum reproductive hormones in the female rats are depicted in Table 5. At 15 days, the extract produced increase in serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone concentration compared with the control.

However, the extract did not produce any significant effect on the serum prolactin concentration. At 30 days, compared with the control, the extract produced increase in serum FSH, LH, estradiol, progesterone and prolactin in both treated female rats.

Table 4. Effect of *C. ferruginea* on the lengths of the left and right uterine horn.

	15 Days		30 Days	
Treatment groups	Length of RUH (cm)	Length of LUH (cm)	Length of RUH (cm)	Length of LUH (cm)
Control	4.717±0.030	4.600±0.063	4.700±0.325	4.817±0.216
$AECF_{50}$	5.150±0.099*	5.350±0.099***	5.817±0.188*	5.733±0.125**
$AECF_{100}$	5.533±0.140***	5.733±0.016***	5.333±0.160	5.417±0.170

Values are means ± SEM (n=6); * = $p<0.05$; ** = $p<0.01$; ***= $p<0.001$. RUH: Right Uterine Horn; LUH: Left Uterine Horn, Control: Distilled water, $AECF_{50}$: Aqueous Extract of *C. freruginea* (50 mg/kg of body weight), $AECF_{100}$: Aqueous Extract of *C. freruginea* (100 mg/kg of body weight).

Discussion

Effects of *C. ferrugina* on estrous cycle

The estrous cycle is the result of cyclical changes in the structures of the vaginal epithelium under the influence of endogenous estradiol. It should be noted that estradiol is a steroid hormone secreted by the ovaries and vagina is a target organ.

It is responsible for the cornification of vaginal mucosa cells causing phases of proestrus and estrus **(**Hubscher *et al*, 2005; Russell, 2008; Freeman, 2008).

The study of the estrous cycle in rat is based on the determination of the different phases by vaginal smears. According to Marcondes *et al.* (2002), the estrous cycle is divided into four phases: proestrus, characterized by the abundant presence of nucleated epithelial cells; estrus with a predominance of keratinized cells (cornified cells); metoestrus in which we observe the same proportion of nucleated epithelial cells and leukocytes; diestrus marked by abundant presence of leukocytes.

Table 5. Effect of *C. ferruginea* on some reproductive hormones of female rats.

Hormones	15 Days			30 Days		
	Control	$AECF_{50}$	$AECF_{100}$	Control	$AECF_{50}$	$AECF_{100}$
FSH (mUI/mL)	5.317±0.359	8.233±0.618**	8.450±0.759**	6.133±0.326	9.800±0.633**	10.200±0.706***
LH (mUI/mL)	9.767±0.486	10.630±0.539	12.450±0.434**	11.350±0.381	13.250±0.521*	14.100±0.584**
Estradiol (pg/mL)	84.222±5.260	100.600±0.972*	101.300±2.863**	107.500±3.428	135.800±6.315**	134.900±5.420**
Progesterone (ng/mL)	45.970±1.915	75.850±11.470*	65.020±6.904	55.850±4.486	74.600±7.593*	81.400±6.480*
Prolactin (mUI/mL)	13.920±0.636	14.780±0.399	15.120±0.324	14.870±1.232	21.170±1.108*	24.700±2.270**

Values are means ± SEM (n=6); * = p<0.05; ** = p<0.01; ***= p<0.001. Control: Distilled water, $AECF_{50}$: Aqueous Extract of *C. freruginea* (50 mg/kg of body weight), $AECF_{100}$: Aqueous Extract of *C. freruginea* (100 mg/kg of body weight).

In this study, daily vaginal smears carried out during treatment with aqueous extract of *C. ferruginea* (50 and 100 mg/kg body weight for 15 and 30 days) revealed a disruption of estrous cycle marked a sharp increase in the number of peaks estrus at the expense of diestrus phases.

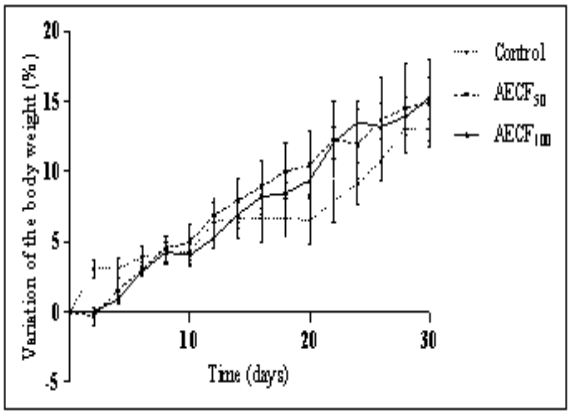

Fig. 1. Evolution of body weight of female rats during treatment with extract of *C. ferruginea* Value are presented as means ± SEM (n=6). Control: Distilled water, $AECF_{50}$: Aqueous Extract of *C. freruginea* (50 mg/kg of body weight), $AECF_{100}$: Aqueous Extract of *C. freruginea* (100 mg/kg of body weight).

In addition, extract induces estrous cycle block in estrus phase of 100% of treated rats until 30th day of treatment. There was also a significant increase in the duration of the stage of estrus at the expense of phases of metestrus and of diestrus which significantly decreases. The slight increase in the duration of the proestrus phase in the treatment of 30 days for both doses versus control was not statistically significant.

The observed effects on the estrous cycle are similar to those of the repeated administration of 17-β-estradiol at a dose of 20.10^{-3} mg/kg of body weight in adult female rat (Kouakou, 2000). These results also corroborate those obtained by Bleu *et al.* (2012) with a daily administration for 28 days of hexanolic, methanol and aqueous extracts of *Passiflora foetida* (Passifloraceae) in adult female rats.

The effects observed on the oestrous cycle are different from those obtained by Kouakou (2000) at the time of the studies of two edible mushrooms (*Daldinia concentrica* (Xylariaceae) and *Psathyrella efflorenscens* (Coprinaceae) recognized for their anti-fertility effects.

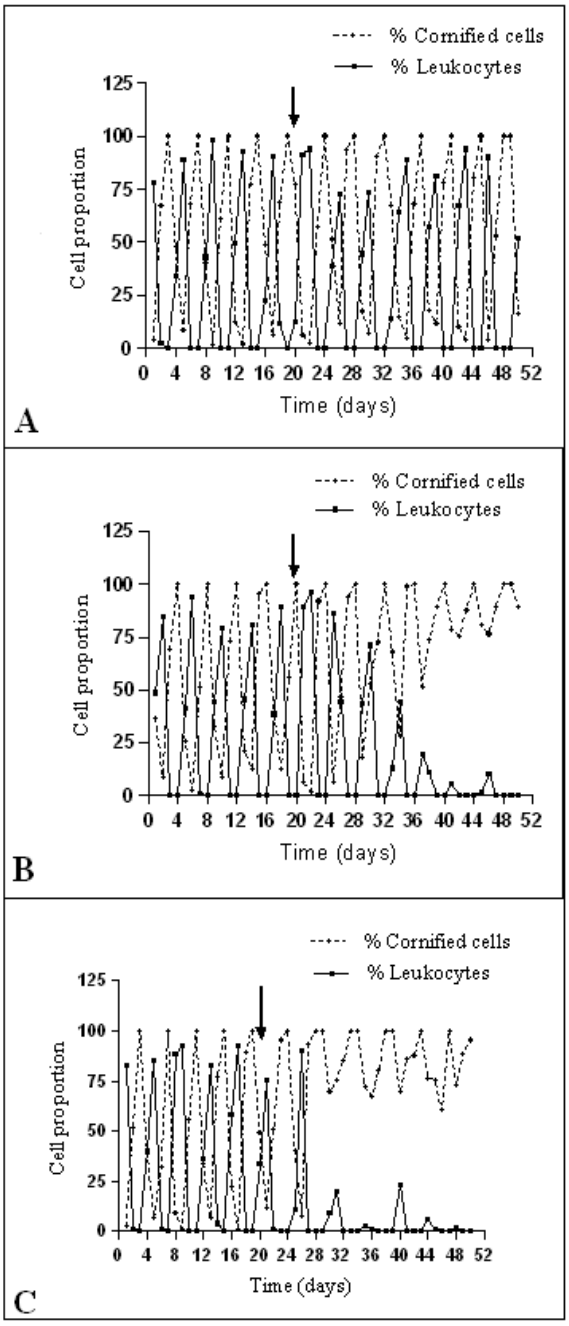

Fig. 2. Evolution of the percentages of the cornified cells and leukocytes obtained before and during 30 days treatment. **A:** Control female rat (Control); **B:** Aqueous extract of *C. ferruginea* 50 mg/kg of body weight ($AECF_{50}$); **C:** Aqueous extract of *C. ferruginea* 100 mg/kg of body weight ($AECF_{100}$). The arrow indicates the beginning of the treatment.

The aqueous extract of *C. ferruginea* could contain substances estrogen-likes or phytoestrogen from where its presence in the organism could mime the activity of the endogenous estrogen.

Effects of C. ferrugina on reproductive organs

The development and functioning of reproductive organs depend on the endocrine system. Indeed, in the female estradiol and progesterone are hormones that ensure the maturation and maintenance of the genital organs. The secretion of these hormones is regulated by pituitary gonadotropin hormones (FSH and LH) themselves under the control of hypothalamic secretion (gonadotropin releasing hormone) (Brann *et al*., 1995).

The effect of repeated administration of extract was measured on the weight of ovary, uterus, cervix and the adrenal gland.

Effects on ovary wet weight

The aqueous extract of *C. ferruginea* produced only a significant increase in wet weight of the ovary after 15 days of treatment with the dose of 50 mg/kg of body weight. For both doses, animals treated for 30 days showed a significant increase in the wet weight of their ovary.

This increase is much pronounced in rats treated with 50 mg/kg of body weight. These results are similar to those obtained by Bleu *et al*. (2012) with hexane and aqueous extracts of *Passiflora foetida* administered to adult rats for 14 and 28 days. Similar results were obtained by Lilaram and Nezzar (2012) and Oyeyemi *et al* (2015) respectively when administered doses of 300 mg/kg of body weight of ethanol extract of *Caesalpinia bonducella* (Caesalpiniaceae) and 600 mg/kg of body weight of aqueous extract of *Momordica charantia* (cucurbitaceae) to rats. This significant increase in ovary weight observed in this study could be explained by strong stimulation of ovary activity by the aqueous extract of *C. ferruginea*. Indeed, the ovarian cycle is marked by folliculogenesis and steroidogenesis under the influence of pituitary gonadotropins (FSH and LH) (Young *et al*., 1999; Gayrard, 2007; Hodgen, 1989; Monniaux *et al*., 2009). During the ovarian cycle, there is a growth of the oocyte, a gradual increase in follicle size and layers of granulosa cells. One also observes the establishment of internal and external theca and fluid-filled cavities (*antrum*) whose volume increases gradually to the stage of Graafian follicle.

The maximum of these phenomena are in phase of proestrus where the level of gonadotropin is at its maximum and resulting in an increase in ovaries weight (Young *et al.*, 1999; Haim *et al.*, 2003; Freeman, 2008).

Thus aqueous extract of *C. ferruginea* could act as these gonadotropins or like molecules stimulating the pituitary to cause release of pituitary gonadotropins which is the cause of the increase in weight of the ovary.

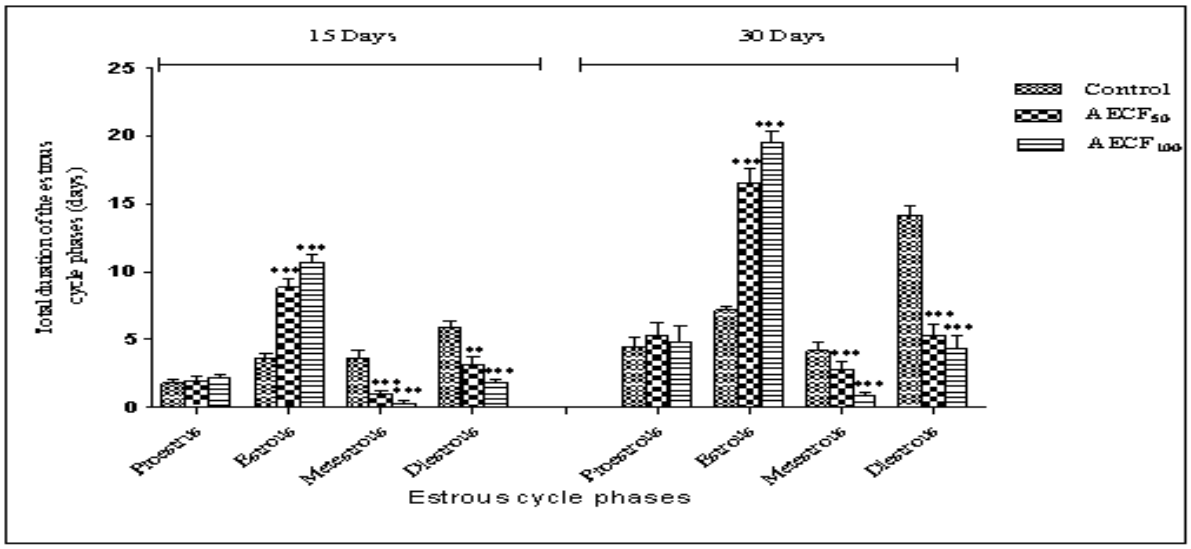

Fig. 3. Effect of the extract of *C. ferruginea* on the duration of different phases of the treated animals. Value are presented as means ± SEM (n=6); **=p<0.01; ***=p<0.001. Control: Distilled water, $AECF_{50}$: Aqueous Extract of *C. freruginea* (50 mg/kg of body weight), $AECF_{100}$: Aqueous Extract of *C. freruginea* (100 mg/kg of body.

Repeated administration of estrogen in intact rats or hypophysectomized causes an increase in ovary weight by stimulating follicle growth. Aqueous extract of *C. ferruginea* may contain estrogenic substances or phytoestrogenic inducing its direct action on the ovary.

Effects on uterine horns wet and dry weight

On the uterine horns, the EACF induces a significant increase of wet weight at a dose of 50 mg / kg body weight for both treatments with an abundance of fluid within the horn. This observation was also made by Bleu (2013) after the administration of methanol extract of *Passiflora foetida* (Passifloraceae) to adults female rats.

These effects are also identical to those of Kouakou (2000), after administration of 17β-estradiol (20.10^{-3} mg/kg of body weight) to normal female rats. Indeed, the increase in uterine weight is an early marker of the basic female for a sufficient exposure to estrogen agonists.

Thus, during the estrous cycle, estrogen and progesterone promote the thickening of the uterine endometrium following cell hyperplasia, an important development of uterine glands which hypertrophies, becomes tortuous and mitotic activity of stroma favoring water imbibition in the tissues and the light of the uterus.

This phenomenon also causes microvascular permeability mediated by growth factors (Kanno *et al.*, 2003b; Chearskul *et al.*, 2004; Russell, 2008). The maximum of this is achieved in phase of proestrus and estrus. All this reaction begins with the essential interaction of estrogen with a high affinity receptor into the uterine tissue. The extract of *C. ferruginea* may contain estrogen-like substances that interact with specific estrogen receptors and would mimic the effects of this substance.

The increase in dry weight of the uterine horns at a dose of 50 mg / kg body weight is contrary to those observed by Kouakou (2000)

when he administered extracts of *Daldinia concentrica* (Xylariaceae) and *Psathyrella efflorescens* (Coprinaceae) two antifertilisants fungi to normal female rats.

Besides the phenomena mentioned above for the increase in wet weight, increasing the dry weight of the uterine horns could be explained by increased stimulation of protein synthesis. The extract could also act in this direction.

Effects on right and left uterine horns

On the length of the right and left uterine horns, the aqueous extract of *C. ferruginea* induced a significant increase at doses of 50 and 100 mg/kg body weight after 15 days of treatment. For the 30 days treated female rats, only the 50 mg/kg of body weight caused a significant increase of both uterine horns.

These results are contrary to those obtained by Oyeyemi *et al.* (2015). Indeed, these authors administered 300 and 600 mg of leaves extract of *Mormodica charantia* (Cucurbitaceae) in normal rats and achieved a significant reduction in the length of left uterine horns.

These results are consistent with those obtained by Raji *et al.* (2012) with regard to the right uterine horns. But these results are contrary when it comes to left uterine horns. These authors found that administration of aqueous extract of *Allium sativum* (Liliaceae) causes a significant increase in the length of right uterine horns and a significant decrease of the length of the left uterine horns.

The significant increase in both uterine horns observed in this study could be explained by an action of the extract of *C. ferruginea* on the growth of this organ.

Effects of *C. ferrugina* on reproductive *hormones*

Effects on pituitary hormones

The results of this study indicated significant increase in pituitary hormones (FSH, LH and prolactin) concentration of treated rats compared with controls. This suggests that the extract could act on the hypothalamus to stimulate the release of GnRH or to synthesis GnRH receptor.

The extract could also stimulate directly the pituitary gland to secrete FHS and LH.

Furthermore, it is recognized that estrogen contribute to the release of GnRH and pituitary hormones (FSH, LH, PRL) through stimulation of its ERα and ERβ receptors located in the hypothalamus and pituitary gland of rats (Wise *et al.*, 1981;. Shupnik and Rosenzweig, 1991). Thus *C. ferruginea* extract may contain estrogen-like substances that would set on ERα and ERβ receptors and endogenous estrogen thought to mimic its activity.

Prolactin helps to initiate breast development by inducing lobulo-alveolar growth of the mammary gland. It also stimulates lactogenesis. Dopamine serves as the major-inhibiting factor or break on prolactin secretion (Fitzgerald *et al.*, 2008). The enhanced level of prolactin observed in this study may be attributed to the effect of the extract probably acting as a dopamine antagonist.

These results are similar to those obtained by Blue *et al.* (2012). Indeed, these authors showed that daily administration of aqueous extract of *Passiflora foetida* at a dose of 500 mg/kg of body weight induced a significant increase in pituitary hormones. Onyegeme-Okerenta *et al.* (2015) also reported that the extract of *Millettia aboensis* (Fabaceae) induced significant increase in pituitary hormone when administered to rats.

Effects on ovarian hormones

This study also showed a significant increase in serum of ovarian hormones (estrogen and progesterone) compared to controls. These results were different from those obtained with the antifertility plant *Millettia aboensis* (Onyegeme-Okerenta *et al.* 2015) which were found to reduce estrogen and progesterone level in serum. Indeed, these hormones are produced by two types of steroidogenic cells (theca interna cells and granulosa cells) of ovarian follicles under the regulatory influence of gonadotropin hormones.

FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells. LH stimulates production of androgen in C19 by acting on receptor located on the theca. Those are aromatized in oestrogens in granulose cells which contain the aromatase (Young *et al.*, 1999; Freeman, 2008; Monniaux *et al.*, 2009). Thus the significant increase observed of FSH and LH could be decisive in the production of these hormones. Extract of *C. ferruginea* may also act directly on the granulosa cells in the ovary by activating aromatase activity. These results corroborate those obtained by Bleu *et al.* (2012) after administration of aqueous extract of *P. foetida* to adult female rat.

The significant increase in serum estrogen confirms the results of the significant increase in the weight of reproductive organs and vaginal smears by blocking all treated females in estrus phases.

The significant increase in progesterone could be explained by the increase in serum LH generated by the extract. Indeed, LH stimulates ovulation and promotes the conversion of the ovulating follicle into the corpus luteum capable to secrete large amounts of progesterone (Niswender *et al.*, 2000). LH also acts on the granulosa cells to secrete progesterone (Christenson and Stouffer, 1997).

The action of the extract may also be direct on the ovary by stimulating the granulosa cells through the receptor of LH. The extract may therefore mimic the stimulatory activity of LH on the granulosa cells to produce progesterone. These results are contrary to those obtained by Yakubu *et al.* (2008) after administration of aqueous extract of *Cnidoscolous aconitifolius* (Euphorbiaceae) an antifertility plant. The effects of *C. ferruginea* may be due to its phytochemical constituents such as alkaloids, flavonoids, isoflavone glycoside (Parvez and Rahman, 1992; Yakubu *et al.*, 2011) substances which are known for their estrogenic effects (Baker *et al.*, 1999; Nazrullaev *et al.,* 2001; Diel *et al.,* 2004).

Conclusion

The pharmacological study of the aqueous extract of *C ferruginea* revealed a blockage of the estrous cycle in estrous and a significant increase in the weight of the reproductive organs and the serum concentration of reproductive hormones. The extract could contain estrogenic compounds favourable to fertility optimization.

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