Journal of Complementary and Alternative Medical Research
2(4): 1-8, 2017; Article no.JOCAMR.31664

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Piper guineense Leaf Extract Elevates Serum Follicle Stimulating Hormone Level in the Diestrus Phase in Non-Pregnant Female Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOA designed the study, managed the literature searches and wrote the protocol. Authors EOA and PCO wrote the first draft of the manuscript. Author CJN managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2017/31664
Editor(s):
(1) Franciszek Burdan, Experimental Teratology Unit, Human Anatomy Department, Medical University of Lublin, Poland and Radiology Department, St. John's Cancer Center, Poland.
Reviewers:
(1) Ayah R. M. Hilles, International Islamic University Malaysia, Malaysia.
(2) Sachin K. Jain, IPS Academy College of Pharmacy, Indore RGT University, Bhopal, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/18632

Original Research Article

Received 19th January 2017
Accepted 4th March 2017
Published 13th April 2017

ABSTRACT

Piper guineense plant has been reported to stimulate reproductive hormone secretion in males; although no known research in females, it is commonly used in the making of postpartum tonics that contract uterine muscles in order to expel clots after birth. Clomiphene citrate was employed in order to ascertain degree of fertility induction and test the effect of methanol extract of *Piper*

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guineense leaf on follicle stimulating hormone (FSH), luteinizing hormone (LH) and oestrogen in non-pregnant wistar albino rats.

Twenty four non-pregnant wistar albino rats on metestrus phase of the estrous cycle were selected and randomly divided into four groups A - D. Group A served as control. Group B rats were administered 1 mg/kg/day of clomiphene citrate only while Group D rats were administered 1 mg/kg/day of clomiphene citrate plus 200 mg/kg/day of *Piper guineense* leaf extract. Groups C rats received 200 mg/kg/day of *Piper guineense leaf* extract only. All administration was via oral route and the experiment lasted for 14 days.

At the end of the experiment, results showed Groups A, B and C rats were in diestrus phase while group D in proestrus. Results also showed statistically significant increase ($P < 0.05$) in plasma FSH in Group C and Group D compared to control group A and Group B. LH and oestrogen were significantly reduced ($P < 0.05$) in Groups B and D compared to control group A and Group C, although LH was significantly higher ($P < 0.05$) in Group D compared to Group B. LH and oestrogen levels remained unchanged in Group C compared with group A.

The results revealed that extract caused an increase in FSH production and steadied LH and oestrogen levels in the serum while combination of extract and clomiphene citrate caused an increase in FSH production as well as significant reduction in LH and oestrogen. FSH remained unaffected in clomiphene citrate treated rats only. The estrous cyclic changes remained unaffected in extract treated rats.

The findings suggest that *Piper guineense* leaf extract caused a significant rise in FSH without altering LH, oestrogen and the estrous cycle in female rats.

Keywords: Piper guineense; clomiphene citrate; gonadotropins; oestrogen.

1. INTRODUCTION

In Nigeria, traditional plants are widely used in induction of fertility and have aroused growing area of interest in the use of traditional plants in induction of fertility because of some side effects and ineffectual action of some fertility drugs. Plants like *Listea chinensis* and *Ochis maculate* have been used to treat male infertility [1]. The present study employed *Piper guineense,* a tropical herbaceous climber plant from Piperaceacea family, that grows up to 20 m tall characterized by heart-shaped leaves and oval, petiole, alternate, 12 cm long [2], usually found in wet places where it supports itself on other plants by means of adventitious roots which are produced along the stem. In Nigeria, it is locally called "*Uziza"* by the Ibos, "*Ata-iyere"* by the Yorubas and "*Mansoro"* by the Hausas. Research has shown that the phytochemical compounds in *Piper guineense* leaf include flavonoids, saponins, tannins, resins, essential oils (such as dillapiol, elemicin, myristicine, and safrole), alkaloids (such as piperine, dihydropiperine wisamine, and dihydowisamine), cardiac glycosides and beta-carophyllene [3-5] which contribute to its medicinal properties [6].

The dried fruits and leaves are commonly used in Southern Nigeria to make postpartum tonics in order to enhance uterine muscle contraction for expulsion of debris from the uterus due to its cholinergic activity [7,8], and also enhanced testicular hormone secretion [9]. Apart from reproductive functions, *Piper guineense* extract has been reported as anticonvulsant [10], molluscicidal [11], hepatoprotective [12], and improved hematological parameters [13].

However, clomiphene citrate is the treatment of first choice in the management of infertility in normally oestrogenized- annovulatory women [14]. It has antiestrogenic effect on hypothalamus therefore causing an increase in the levels of gonadotropin-releasing hormone which in turn causes in increased secretion of FSH and LH that result in ovulation [15].

However, there is no known scientific article on the effects of *Piper guineense* plant on female reproductive hormone to the best of our knowledge. Here the present study compared the effects of *Piper guineense* leaf extract and clomiphene citrate on plasma FSH, LH, and oestrogen levels.

2. MATERIALS AND METHODS

2.1 Experimental Design

Twenty-eight non-pregnant female adult wistar rats weighing 150 - 230 g and 9 weeks old were used in the study. The animals were obtained from the Animal House of Department of Pharmacology, College of Medicine and Health Sciences, University of Port Harcourt, Nigeria

and transported to Madonna University Nigeria. They were kept in standard cages (Henan, China) and acclimatized for 2 weeks in the Animal House, Department of Physiology, Madonna University (Room temperature of 40 ℃). They had access to normal rat food and water *ad libitum*. The basal body weight of rats was taken before administration of drug and/or extract. The rats' vaginal smears were examined between 9:00 - 11:00 am of the first day. Twenty six female rats on metestrus phase of estrous cycle were uniformly selected. Twenty four rats were randomly divided into 4 groups A - D (n = 6). Group A (control) rats were given tap water and rat chow. Groups B, and D rats were administered 1 mg/kg/day of clomiphene citrate respectively as described by Boyar et al. [16]. Group C and D rats were administered 200 mg/kg/day of *Piper guineense* leaf extract respectively as described by Nwozo et al. [12]. The experiment lasted for 14 days. All administration was done orally using gavage.

The vaginal smears of rats were continuously examined daily between 9:00 to 11:00 am throughout the duration of study. The estrous cycle was determined in accordance with Marcondes et al. [17].

2.2 Drug and Extract Preparation

2.5 g of clomiphene citrate tablet (Doppel Pharmaceuti, Italy) (50 mg per tablet) was purchased over the counter at Orchard Pharmacy store in Owerri, Imo State and crushed into a powdered form. The powdered form was suspended in methanol (Sigma Aldrich, USA) and filtered with whatman paper (no. 1) to remove excipients. The filtrate was administered as clomiphene citrate.

The fresh leaves of *Piper guineense* were purchased from the central market in Elele, River State, Nigeria. They were authenticated in the Department of Pharmcognosy, Madonna University Nigeria with voucher number (MUE/PGSY/004). 550 g of *Piper guineense* leaves were sun-dried and ground in powdered form. The powdered form was weighed in a Laboratory balances (Grainger). 226 g was extracted with 300 ml ethanol using Maceration extraction method by Sukhdev et al. [18] for 24 hours in an air tight container. Using a vacuum rotary evaporator (Buchi), the ethanol filtrate was concentrated at a low temperature, under reduced pressure. This yielded 43.03 g (19.0%) (jelly-like) oil extracts, 25 g of which was then dissolved in 5 ml methanol stored in a refrigerator from which fresh solution was prepared using distilled water.

2.3 Hormone Measurement

At the end of the 2 weeks administration, the rats were subjected to urethane inhalation anaesthetic with cotton wick in a chamber. 5 ml of blood was collected from rats via cardiac puncture and stored in well labeled EDTA bottles for hormone measurement. FSH, LH, and estrogen were measured using Enzyme Linked Immuno-SorbentAssay (ELISA) method.

2.4 Statistical Analysis

Results were expressed as mean ± standard error of mean (SEM). Statistical significance of differences observed between Control and Experimental groups was analyzed using one way analysis of variance (ANOVA). Any significant ANOVA was further analysed by Tukey's post hoc test using SPSS version 18. P values < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

There was no record of death during and after drug and extract administration. The food intake and water consumption of the rats administered drug and/or extract was not affected during the experiment. The change in body weight was not recorded although it has been shown that rats treated with *Piper guineense* extract increased in body weight [19].

Fig. 1a showed changes from metestrus on day 1 in control group A, Groups B and C rats to diestrus on day 14, whereas Group D changed from metestrus on day 1 to proestrus on day 14. The duration of the estrous cycle was shortened in Group D rats administered both clomiphene citrate and extract, while the estrous cycle of Groups B and C administered clomiphene citrate and extract respectively remained unaffected.

Table 1 showed statistically significant increase (P < 0.05) in FSH levels in Groups C and D rats (0.36 ± 0.14, and 0.50 ± 0.07) compared to control group A and Group B rats (0.17 ± 0.01 and 0.14 ± 0.05) respectively while results in Fig. 1b and c revealed statistically significant decrease (P < 0.05) in LH and oestrogen levels of Groups B and D rats (0.20 ± 0.15; 28.36 ± 11.22, and 0.43 ± 0.03; 28.49 ± 14.76) compared

to control group A and Group C rats (0.88 ± 0.91; 57.07 ± 14.99 and 0.92 ± 0.11; 52.33 ± 19.35).

Normally the gonadotropins (LH and FSH) are secreted from the anterior pituitary, regulated by the gonadotrophin-releasing hormone which is produced and released in rhythmic pulsatile fashion [20] into the median eminence and secreted into the hypothalamic-hypophyseal portal system to the anterior pituitary to trigger FSH and LH release [21]. The effects of these gonadotropins stimulate normal follicular growth and ovulation [22]. In female rats, the release of FSH and LH varies during reproductive stages [20]. FSH and LH levels decrease during diestrus II and begin to rise in the late proestrus phase [23]. Taking this fact into consideration that the FSH levels was significantly reduced in the control group A rats in the same diestrus phase as Group C rats suggest that *Piper guineense* leaf extract caused the rise in the serum FSH level.

On the other hand, FSH level in the clomiphene-treated Group B rats was expected to increase due to usual antiestrogenic activity of clomiphene citrate [15] that mediate triggering of gonadotropin secretion from the pituitary. It appeared the estrous cycle influenced clomiphene citrate effect on the hypothalamic gonadotropin-releasing hormone in Group B thereby affecting pituitary secretion of FSH. Nevertheless the increased FSH level in Group C rats would be expected to be associated with an increase in estrogen and LH levels because FSH is principally responsible for follicular growth and estrogen formation by inducing aromatase enzyme that converts androgen to estradiol [24]. Meanwhile, the LH and estrogen levels remained normal in *Piper guineense* Group C rats irrespective of the rise in the FSH. It has been reported that low estradiol levels inhibit release of gonadotropin-releasing hormone in the early stages of the estrous cycle in rats until estradiol surge during proestrus that gonadotropin-releasing hormone is released thus causes the release of LH [25]. Based on this report, LH and estrogen levels in the control group A and Group C rats would be regarded as high levels of LH and estrogen levels respectively since LH and estrogen secretion occurred during diestrus phase in the present study. More so, LH and estrogen levels in the proestrus Group D rats would be expected to markedly increase compared to normal control and Group C rats but since the LH and estrogen levels in Group D did not rise strongly suggest the presence of anti-luteinizing hormone and anti-estrogenic effect in either clomiphene citrate or the extract. Our recent findings showed that LH and estrogen levels markedly increased in clomiphene citrate treated rats and markedly reduced in rats treated with clomiphene citrate and *Tetrapleura tetraptera* extract during proestrus phase of the estrous cycle [26] inferring that the synergy negatively influenced LH and estrogen production. And now it is evident that the synergy between *Piper guineense* and clomiphene citrate resulted in the reduced levels of LH and estrogen. Data have shown that *Tetrapleura tetraptera* extract inhibited LH and estrogen in vivo and in vitro [26,27]. These reports strongly showed that whether clomiphene citrate is combined with extracts that enhance or inhibit LH secretion would result in low levels of circulating LH. Normally estradiol regulates the functions of the hypothalamic-pituitary axis in a feedback manner although inhibin is a major regulator of FSH secretion during the estrous cycle of the rat [28]. High titre of estradiol in the peripheral circulation will lead to a negative feedback to the hypothalamus resulting in a decreased gonadotropin-releasing hormone associated with reduced FSH and LH secretion from the anterior pituitary. It can be stated that since oestrogen level of Group C remained in parity with the normal control rats, FSH levels would be expected to remain unaffected. Increased FSH recorded would be expected to trigger folliculogenesis associated with high titre of estrogen. The estrogen in a feedback manner would stimulate pituitary production of LH. However, the increase in FSH with normal levels of LH and estrogen in Group C rats treated with extract only during diestrus phase could only suggest that the extract acted directly on the anterior pituitary although other unknown mechanisms could be responsible. Data have shown that active substance piperidine derivatives from *Piper guineense* cross the blood-brain barrier and enhance serotonin and catecholamine metabolism [29] indicating the possibility of the extract action on the anterior pituitary gonadotropes to cause increase in FSH independent of estradiol or ovarian regulation. In addition, other possible mechanisms could implicate *Piper guineense* extract attenuating rhythmic pulsatile release of the hypothalamic gonadotropin-releasing hormone which is central to the corresponding pulsatile release of the pituitary LH into the peripheral circulation [20]. However, in male animals, *Piper guineense* has been showed to cause significant increase in testosterone in semen and testes in rats [19] and

mice [30]. Data have also shown 10 mg of piperine (active component in *Piper guineense*) caused marked increase in serum gonadotropins in mature male albino rats [31]. The present study implies that the increased level of FSH could result in early menopause because elevated level of FSH has been associated with diminished ovarian reserve in clinical studies [32].

Table 1. The effects of *Piper guineense* leaf extract and clomiphene citrate on FSH, LH and oestrogen in non-pregnant wistar albino rats

Groups	FSH (mIU/ml)	LH (mIU/ml)	Oestrogen (pg/ml)
Group A	0.17 ± 0.01	0.88 ± 0.91	57.07 ± 14.99
Group B	0.14 ± 0.05	0.20 ± 0.15*	28.36 ± 11.22*
Group C	0.36 ± 0.14*	0.92 ± 0.11	52.33 ± 19.35
Group D	0.50 ± 0.07*	0.43 ± 0.03*	28.49 ± 14.76*

Data is represented as Mean ± SEM; (*) denotes P < 0.05 statistically significant compared to group A (control)

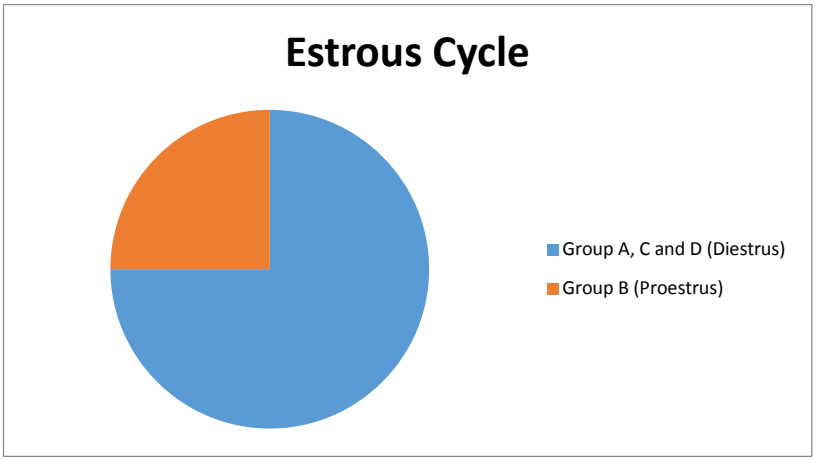

Fig. 1a. The estrous cycle phases in non-pregnant wistar albino rats at the end of 14 days experiment

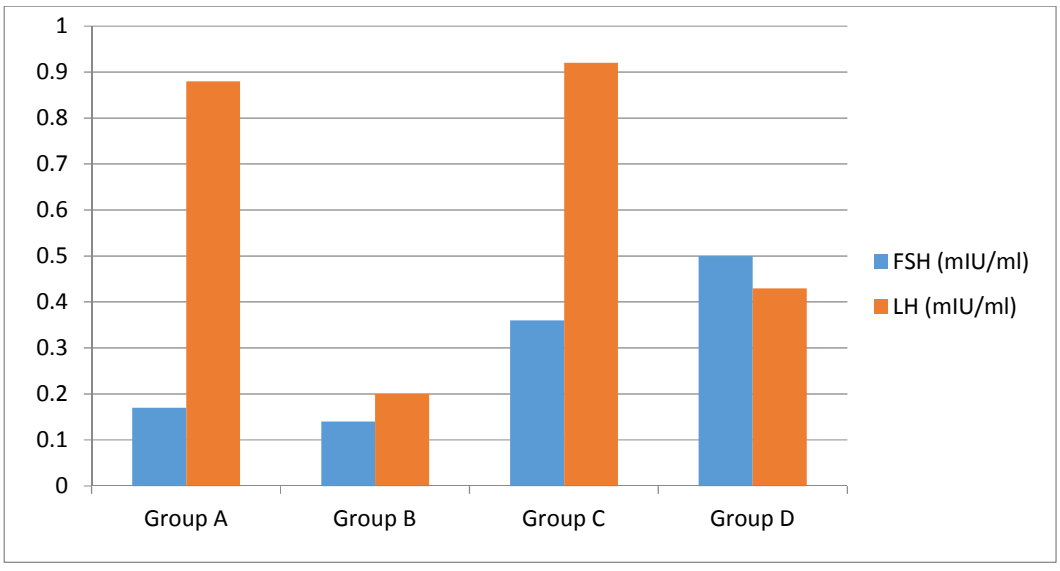

Fig. 1b. The effects of *Piper guineense* leaf extract and clomiphene citrate on FSH and LH in non-pregnant albino wistar rats

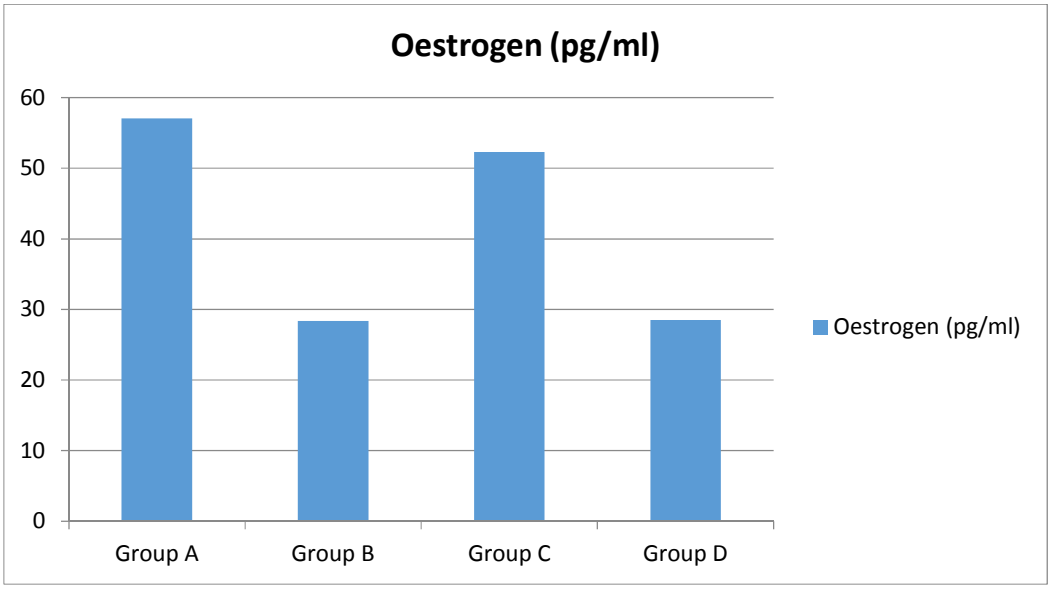

Fig. 1c. The effects of *Piper guineense* leaf extract and clomiphene citrate on oestrogen level in non-pregnant albino wistar rats

4. CONCLUSION

The present study concludes that *Piper guineense* leaf extract mediated increased FSH production during diestrus phase, without affecting the LH and estrogen levels therefore suggesting that 200 mg/kg of *Piper guinnense* extract can expedite early follicular phase resulting in diminishing the population of follicles in the ovary. Although at the moment we cannot ascertain actual mechanisms responsible for the increased FSH therefore further studies would be recommended.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all the experiments followed standard protocol and was approved by Research Committee, Department of Human Physiology, Madonna University Nigeria, headed by Agbai E. O (Acting Head of Department) and by Professor A. C. Ugwu (Sir) of Department of Human Physiology, University of Benin, Edo State, Nigeria as an External Examiner to the Department of Human Physiology, Madonna University Nigeria. All experiments have therefore been performed in accordance with the ethical standards laid down in 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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