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Comparison of inhibitory effect of ibuprofen with *Piper guineense* Schumach and Thonn. on some reproductive hormones in female rats

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ABSTRACT

The aim of the present study was to compare the inhibitory effect of ibuprofen with oral administration of *Piper guineense* leaf extract on follicle-stimulating hormone, luteinizing hormone, progesterone and estrogen in female rats irrespective of the estrous cycle. The animals were randomly assigned to four groups (n = 7): group A (control), Group B, 180 mg^{-kg} of ibuprofen, Group C, 200 mg^{-kg} of *Piper guineense* extract, Group D, 180 mg^{-kg} of ibuprofen and 200 mg^{-kg} of *Piper guineense* extract. At the end of two weeks administration, rats were sacrificed under urethane anesthesia and hormones measured using enzyme-linked immunosorbent assay method. Results showed significant reduction in serum follicle-stimulating hormone and luteinizing hormone following ibuprofen administration in Group B rats at $P < 0.05$. *Piper guineense* extract treated Group C rats caused significant reduction in serum luteinizing hormone and progesterone at $P < 0.05$. In contrast, serum follicle stimulating hormone significantly increased in Group D rats at $P < 0.05$ whereas serum luteinizing hormone and progesterone were markedly reduced at $P < 0.05$. Serum estrogen level remained unchanged among groups. In conclusion, results obtained suggested that extract inhibited luteinization of follicles thus could impair ovulation, therefore the extract can be used as oral contraceptive in family planning.

Keywords:estrogen, follicle-stimulating hormone, ibuprofen, luteinizing hormone, Piper guineense, progesterone.

INTRODUCTION

Reproductive hormones are central to normal female ovulatory cycle. The hypothalamic-pituitary-ovarian axis has been shown to control the processes necessary for induction of ovulation and fertility[1]. Because normal ovulation principally determines female fertility[2], therefore alteration in the ovulation processes by hypothalamic-pituitary-ovarian hormone dysfunction could result in infertility. Several plants such as *Tetraptera tetrapleura*, *Mormordica charantia*, *Cuminum cyminum*, *Nelumbonucifera* and *Cnidoscolousaconitifolius* have been shown to alter reproductive hormone secretion thus can be used as contraceptives[3-6].

The plant, *Piper guineense* is commonly used as condiment in the preparation of pepper soups in West African cuisines based on its spicy, flavoured piperine constituent responsible for sexual arousal in rats[7]. The seed and leaf extracts are rich in phytochemical constituents such as saponins and tanins[8]. In Southern Nigeria, *Piper guineense* seed and leaf are used in the preparation of postpartum tonics to enhance expulsion of debris although study has shown that the extract enhances uterine muscle contraction in order to expel debris postpartum[9]. Because the leaves are locally used for female infertility[10] led to our recent study that showed *Piper guineense* leaf extract caused elevation of follicle-stimulating hormone (FSH) level during diestrus phase of estrous cycle[11]. Moreover, increased serum FSH levels has been implicated in impairment of ovarian function associated with decreased number of follicles[12]. These recent findings aroused our interest to investigate its effect randomly and in comparison with ibuprofen.

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) that suppresses prostaglandin synthesis through its inhibition of cyclooxygenase activity although cyclooxygenase-2 is believed to set alarm for ovulatory clock[13, 14]. It has been reported that NSAIDs inhibit ovulation and reduce progesterone levels in young women[15]. Because ibuprofen exhibits inhibitory action on female reproductive hormones thus it becomes a good model of comparison with *Piper guineense.* Therefore, the present study compared the inhibitory effect of Ibuprofen with *Piper guineense* leaf extract on FSH, LH, estrogen and progesterone

levels irrespective of the estrous cycle in female Wistar rats.

MATERIALS AND METHODS

Animals

Twelve weeks old 28 non-pregnant female Wistar rats weighing 250 - 300 g were used in the study. They were obtained from Animal House unit of Department of Pharmacology, University of Port Harcourt, Rivers State and transported to Animal House unit of Department of Human Physiology, Madonna University, Rivers State, Nigeria where they were housed. They were kept in cages (Henan, China) and acclimatized for 2 weeks under room temperature between 27 °C and 33 °C. They had access to tap water *ad libitum* and normal rat chow.

Animals received humane care according to criteria outline in the Guide for Care and the Use of Laboratory Animals prepared by the National Academy Science and published by National Institute of Health[16].

Experimental design

The rats were randomly selected (n = 7) irrespective of their estrous cycle. Group A rats were used as normal control. Group B rats received oral administration of 180 mg^{-kg} of ibuprofen (high dose) as published in Pfizer Data Sheet[17]. Group C rats received oral administration of 200mg^{-kg} of *Piper guineense* leaf extract according to method described by Agbai and Nwanegwo[6, 18]. Group D rats received oral administration of 180 mg^{-kg} of ibuprofen and 200 mg^{-kg} of *Piper guineense* leaf extract simultaneously.

Ibuprofen preparation

Two packets of ibuprofen (Bristol UK) containing 56 coated tablets (400 mg per tablet) were purchased over the counter at pharmacy shop Owerri, Imo State and ground into a powdered form. The grounded form was soaked in ethanol (Sigma Aldrich, USA), sieved using whatman paper and extracted and excipients were carefully collected on the filter paper and removed. The filtrate was considered as ibuprofen.

Piper guineense leaf extraction

Fresh leaves of *Piper guineense* were purchased from Afor Ogbe market in Mbaise, Imo State on 08/06/2015 and were identified in the Department of Pharmacognosy with voucher number (MUE/PGSY/004). The leaves were sorted, cleaned, sun-dried for six days and ground into a coarse powdered form in a mortar. 100 g of the powdered form was collected and suspended in 100 ml of ethanol (Sigma Aldrich, USA) and stirred continuously to make sohxlet mixture. The mixture was filtered using Whatman paper (No. 1). The filtrate was dried with Rotatory evaporator (Buchi) in a semi solid mass and stored in air tight container and kept in a refrigerator at a temperature of 4 °C. The extraction lasted for 2 days.

Hormone measurement

At the end of two weeks administration, the rats were anesthetized with urethane soaked with a cotton wick put in a glass chamber. About 5 ml of blood was collected from the rats via cardiac puncture and stored in a well labeled EDTA bottles to avoid blood coagulation. FSH, LH, estrogen and progesterone levels were measured using Enzyme-linked Immunosorbent Assay (ELISA) method.

Statistical analysis

Results were expressed as Mean ± Standard Error of Mean (SEM). Statistical significance of difference observed between control and experimental Groups was analyzed using one way Analysis of Variance (ANOVA). Any significant ANOVA was analyzed by Tukey's post hoc test using SPSS version 18. P values < 0.05 were considered statistically significant.

RESULTS

Results showed ibuprofen caused statistical reduction ($P < 0.05$) in FSH levels (0.15 ± 0.02 miU^{-ml}) compared to control group A rats (0.34 ± 0.08 miU^{-ml}). *Piper guineense* leaf extract treated Group C rats did not cause any significant difference in FSH level (0.26 ± 0.02 miU^{-ml}) compared with control group A at $P > 0.05$. Ibuprofen plus *Piper guineense* leaf extract treated Group D rats caused statistically significant increase in FSH level (0.54 ± 0.09 miU^{-ml}) compared to control group A rats at $P < 0.05$.

Table 1: The comparison of inhibitory effect of ibuprofen with *Piper guineense* leaf extract on some reproductive hormones

Groups	FSH (miU^{-ml})	LH (miU/ml)	Progesterone (ng/ml)	Estrogen (pg/ml)
Group A	0.34 ± 0.08	1.05 ± 0.41	16.66 ± 0.29	28.00 ± 0.78
Group B	0.15 ± 0.02*	0.33 ± 0.14*	20.71 ± 0.51	35.84 ± 1.62
Group C	0.26 ± 0.02	0.24 ± 0.02*	13.65 ± 0.24*	25.55 ± 0.47
Group D	0.54 ± 0.09	0.32 ± 0.04*	13.12 ± 0.05*	21.82 ± 0.23

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

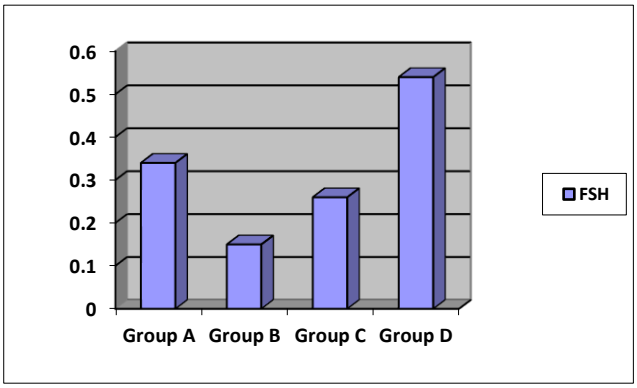

Figure 1: The effects of ibuprofen and/or *Piper guineense* leaf extract on serum follicle-stimulating hormone

There was statistically significant reduction in LH levels ($P < 0.05$) of experimental Groups B, C and D rats (0.33 ± 0.14, 0.24 ± 0.02 and 0.32 ± 0.04) miU^{-ml} compared to control group A (1.05 ± 0.41 miU^{-ml}).

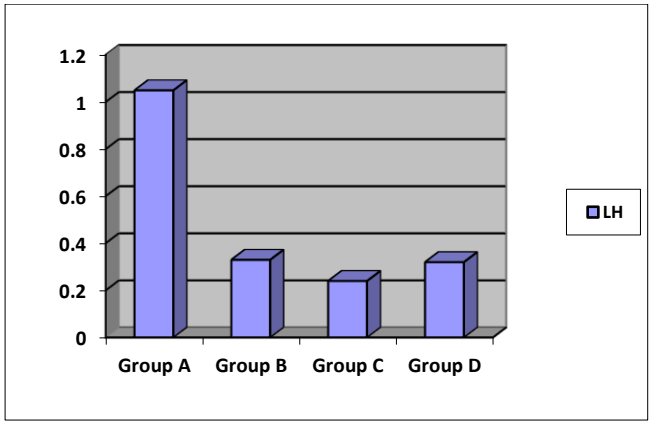

Figure 2: The effects of ibuprofen and/or *Piper guineense* leaf extract on serum luteinizing hormone

There was also statistically significant reduction in ($P < 0.05$) progesterone levels of Group C and D (13.65 ± 0.24 and 13.12 ± 0.05) ng^{-ml} compared to control group A (16.66 ± 0.29 ng^{-ml}). However, there was no statistically significant ($P > 0.05$) between control group A and Group B (20.71 ± 0.51 ng^{-ml}).

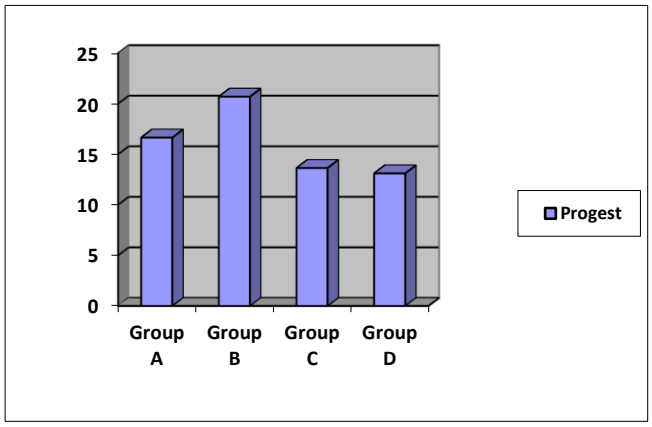

Figure 3: The effects of ibuprofen and/or *Piper guineense* leaf extract on serum progesterone level. (progest = progesterone).

There was no statistically significant difference in estrogen levels ($P > 0.05$) between control group A (28.00 ± 0.78 pg^{-ml}) and experimental Groups B, C and D (35.84 ± 1.62, 25.55 ± 0.47 and 21.82 ± 0.23 pg^{-ml}).

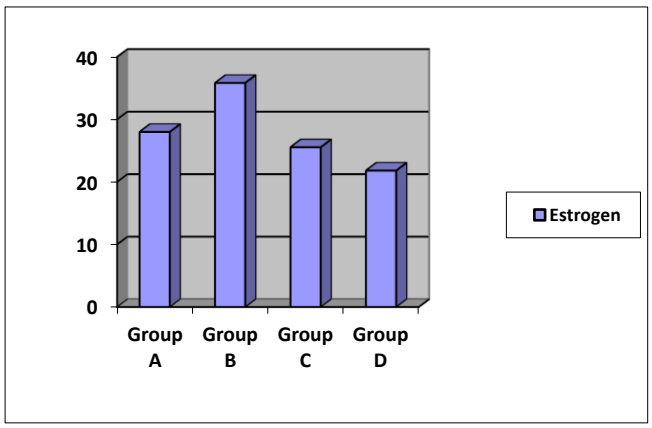

Figure 4: The effects of ibuprofen and/or *Piper guineense* leaf extract on serum estrogen level.

DISCUSSION

Effect of ibuprofen on the serum FSH, LH and estrogen level

As expected, result in Fig. 1 ibuprofen caused significant reduction in FSH and LH levels compared to normal control. Studies have shown that ibuprofen targets cyclooxygenase-2 enzyme responsible for synthesis of prostaglandins[19]. These prostaglandins are inflammatory mediators that trigger follicular rupture and are important in ovulation[14].Studies by Sirois and colleagues have shown the importance of preovulatory LH surge in cyclooxygenase-2 synthesis by the granulosa cells[20, 21]. Since ibuprofen blocks cyclooxygenase-2, it shows that the importance of preovulatory surge is rendered unsuccessful thus resulting in failure in the biosynthesis of follicular prostaglandins which in turn inhibit inflammatory cascade necessary for follicular rupture prior to ovulation[22]. The outcome could result in anovulation. Studies have also shown that anovulation is associated with reduction in estrogen, progesterone and LH peak[23, 24], thus supporting the present result that showed significant reduction in serum LH levels in ibuprofen-treated rats.

Apart from the possible anovulation that could result from ibuprofen administration, FSH was significantly reduced in the ibuprofen-treated Group B rats implying follicle development and maturation could be attenuated because FSH is implicated in the early ovarian cycle processes as it initiates and maintains the development of many antral follicles during which the dominant follicle is selected[25]. Moreover, follicle development and maturation is dependent on LH and FSH[26], since low serum FSH and LH levels was observed suggested ibuprofen could impair follicle development and maturation. Studies have shown that follicular dysfunction or hypothalamic-pituitary-ovarian dysfunction could impair ovulation[27].

Fig. 4 showed estrogen levels remained normal despite the reduction in FSH levels. FSH is not only central in the control of ovarian follicle development butsteroidogenesis processes[28]. FSH binds to granulosa cells and activates aromatase enzyme which converts androgen from theca cells into estrogen and failure to bind results in follicular atresia[29]. Since estrogen level remained normal implied that ibuprofen might not alter steroidogenesis irrespective of the small titre of the serum FSH (0.15 ± 0.02 miU^{-ml}).

Although it is the function of the hypothalamic-pituitary-ovarian mechanism to fine tune the estrogen levels that is characterized by an initial rise in estrogen levels followed by a fall[30], the present result showed that estrogen remained unchanged in the ibuprofen-treated rats compared to control group A rats in the face of ibuprofen-attenuated FSH and LH levels. On the other hand, serum progesterone levels in Fig. 3 also remained normal similar to estrogen in ibuprofen treated rats. Because ibuprofen caused reduced serum LH and FSH levels (Fig. 2) with the possibility of follicle development and maturation impairment and impairment, it is surprising that serum progesterone level remained unchanged because ovulation precedes progesterone production. LH surge has been shown to facilitate progesterone production[31].In regard to the unchanged serum levels of estrogen and progesterone, it appeared that ibuprofen mediated its action on the gonadotropins without attenuating the ovarian hormone secretion. Adrenal glands have been implicated as major sources of progesterone in ovariectomized deer[32],and released in response to stress during low ovarian output follicular phase of menstrual cycle in naturally cycling women[33]. There could be possibility of extra-ovarian progesterone constituting to the serum progesterone pool.

Effect of *Piper guineense* leaf extract on the serum FSH, LH and estrogen level.

In *Piper guineense* extract treated Group C rats, FSH remained normal whereas LH was significantly reduced in contrast with the results of our recent study that showed that the extract caused increased FSH levels and LH peak during the diestrus phase of the estrous cycle[11]. Although the present study evaluated serum FSH and LH levels irrespective of the estrous cycle, the differences in serum FSH and LH levels could be dependent on the estrous cycle since the rats received the same doses of *Piper guineense* leaf extract (200 mg^{-kg}). The hypothalamic gonadotropin-releasing hormone is released into the anterior pituitary via the hypothalamic-hypophysial portal vessel to trigger the release of FSH and LH during the follicular and diestrus phase. The FSH rises prior to luteal/proestrus phase that is characterized by the increased LH that subsequently peaks in the luteal phase. It is obvious that the reduction in serum LH levels is a function of the *Piper guineense* leaf extract.

The reduction in LH levels in extract treated Group C rats was associated with significant reductions in serum progesterone level and normal serum estrogen level compared to control group A rats. It is evident that the reduction in LH could have attenuated follicle development and maturation resulting in anovulation. The granulosa cells mature into corpus luteum following follicle rupture and release of the ovum. The corpus luteum secretes progesterone[34]. It has been reported that progesterone secretion is largely dependent on the LH and FSH[35, 36]. Therefore the failure for the formation of corpus luteum and significant reduction of LH could cause the low level of progesterone in the present study since LH surge is responsible for inflammation of follicle and rupture (Epsey and Lipner, 1994)[37]. Although extra-ovarian progesterone secretion could be implicated but it appeared the extract inhibited progesterone secretion as evident with the low level of progesterone in figure 3. Studies have shown that drugs that reduce progesterone level usually block the conversion pregnenolone to progesterone[38]. The extract may block the conversion of pregnenolone to progesterone although the present study did not measure the pregnenolone level. Nevertheless, studies have shown that flavonoids inhibit 3beta-hydroxysteroid dehydrogenase enzyme[39], responsible for conversion of pregnenolone to progesterone Because *Piper guineense* extract is rich is flavonoids[11, 40], the study therefore suggests that flavonoid content of the extract caused significant reduction in the serum progesterone level.

However, the unchanged serum estrogen level observed in *Piper guineense* Group C correlated with the serum FSH level. The synthesis of estrogen occurs in the ovaries especially in the granulosa cells, theca cells and corpus luteum but corpus luteum is exonerated, therefore, the production of the estrogen solely rests on the granulosa and theca cell. The granulosa cells synthesize estrogen under LH stimulation[41]. Via synthesis and secretion of pregnenolone which diffuses into the theca cells and pregnenolone is converted to androstenedione[42]. The androstenedione in turn reenters into the granulosa cells where it is converted to estrone by aromatase enzyme and subsequently estradiol by 17beta-hydroxysteroid dehydrogenase[43]. Both aromatase enzyme and 17beta-hydroxysteroid dehydrogenase are controlled by the FSH stimulation[36, 44], and normal FSH level proportionate estrogen normal level.

Results of Group D rats treated with *Piper guineense* leaf extract and ibuprofen showed significant increase in FSH. It could be hypothesized that this elevation in serum FSH level could be a summation of inhibitory action of ibuprofen and the action of the extract on FSH secretion. These could lead to disinhibition of gonadotrope and possible hyperstimulation of the gonadotropes thus causing over-production of FSH.As aforementioned ibuprofen blocked FSH and LH levels and could result in the failure to generate cyclooxygenase-2 that leads to biosynthesis of prostaglandins. Prostaglandin plays an important role in follicle rupture thus failure to secrete prostaglandin results in the inability of selected follicle to ovulate[37]. Another plausible reason for the increased serum FSH in Group D rats could involve extragonadal tissue secretion of activin that stimulates FSH biosynthesis and release from the gonadotrope cells via autocrine-paracrine mechanism[45].

On the other hand, significant reduction in serum LH level occurred due to synergistic inhibitory action of both ibuprofen and the extract. The serum estrogen level was not significantly different from the normal control group A and could be based on the actions of ibuprofen and *Piper guineense* leaf extract since both did not cause any significant change in the serum estrogen level. The serum progesterone level was markedly reduced. The reduction could be dependent on the hypothalamic-pituitary dysfunction evoked by both treatments because this dysfunction is often responsible for alteration of ovarian hormone production[46].

In conclusion, the results obtained in this study suggested the use of *Piper guineense* leaf extract in contraception since it caused inhibition of LH and progesterone secretion indicating possible anovulation. This research has also thrown new light on the inhibiting action of ibuprofen on the gonadotropins and the sparing effect on the ovarian hormone secretion.

Limitations

The inhibitory actions of the extract and ibuprofen were not related toproestrus/estrus phase in the randomized experimental animals thus we cannot extrapolate our findings to the potential use of *Piper guineense* extract in contraception.

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