ACUTE EFFECTS OF XYLOPIC ACID ON SEX HORMONES AND LACTATION IN POSTPARTUM FEMALE RATS

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ABSTRACT

The aim of this study was to evaluate the effects of xylopic acid (XA) on sex hormones and lactation in postpartum female rats. Fifteen pregnant female rats were used for the study. They were assigned randomly to three different experimental groups (n=5).The first group which served as control was given distilled water for seven days, while the second and third groups were given low dose of 25mg/kg and high dose of 100mg/kg body weights(BW) of XA respectively, once daily for seven days. In all the groups, treatments were given orally immediately after parturition. Twenty four hours after the last doses, whole blood was collected by cardiac puncture from each animal for the measurements of hormonal profile using enzyme-linked immunosorbent assay (ELISA) kits. Results showed that serum levels of Prolactin and Estrogen were significantly reduced ($P<0.05$) in rats treated with both 25 and 100 mg/kg BW of XA while significant reduction ($P<0.05$) in serum Progesterone and Testosterone occurred only in rats treated with 100mg/kg BW.Xylopic acid caused an observable increase in serum levels of FSH and LH. We therefore conclude that XA possesses inhibitory effects on lactation in postpartum female rats through a mechanism that may be associated with the suppression of ovarian hormones (Estrogen and Progesterone) which consequently results in prolactin inhibition.

INTRODUCTION

Xylopia aethiopica is an angiosperm belonging to the custard apple plant family called Annonaceae (Choumessi *et al.,* 2012). It is an exceptional spice in many African folks because of its great nutritional and medicinal values in traditional medicine. . *Xylopia aethiopica* is vital in African traditional medicine as it is believed to have broad therapeutic indications. It is usually recommended as a postpartum tonic for arresting bleeding among women that put to bed (Nnodim *et al.*, 2011), treatment of cough, biliousness, bronchitis, rheumatism, dysentery, malaria, uterine fibroid, amenorrhea (Burkill, 1995). It has also been reported to promote fertility and ease childbirth (Iwu, 2014; Burkill, 1995). Extracts and isolates from almost all parts of the plant tend to possess one bioactivity or another that confirms its traditional uses, and have largely shown to be of low toxicity (Fetse *et al.,* 2016). However, results of some of the studies on this plant do not agree with some of its traditional therapeutic uses. For example Obembe *et al.,* (2015) revealed that aqueous extract of *Xylopia aethiopica* increases bleeding and clotting time. Most of the studies on this plant are on its crude extract. Recent studies, however, now focus on its active constituents of which xylopic acid is major.

Xylopic acid (XA) is a diterpene kaurane derivative [15-β-acetoxy-(-)-kaur-16-en-19-oic acid] which has been shown to possess analgesic, (Ameyaw *et al.,* 2013) anti-androgenic and spermatotoxic (Woode *et al*, 2012), cardiovascular and diuretic (Somova *et al.*, 2001) activities. Despite the advancement on this plant, its effects on sex hormones and lactation among postpartum women have not been given adequate attention. In the light of this, we evaluated the effect of xylopic acid on sex hormones during lactation in postpartum female rats.

Figure 1: The dried fruit of *Xylopia aethiopica.*

The fruits are small, carpels, forming dense cluster, twisted bean-like pods, dark brown, cylindrical, long and thick; the contours of the seeds are visible from outside (Adapted from Gernot Katzer's spice pages)

Prolactin, is the principal lactogenic hormone critical to the establishment of lactation, milk macronutrient content and milk production. Prolactin is secreted from the pituitary gland and the concentration of circulating serum prolactin level usually increases during pregnancy so that by the end of gestation, levels are 10 to 20 times over normal amounts. Prolactin is prevented from exerting its effect on milk secretion by elevated levels of progesterone. At parturition, progesterone and estrogen levels decrease significantly which result in copious milk secretion by mammary gland. Prolactin levels decrease as lactation is established but nursing stimulates prolactin release from the anterior pituitary gland which promotes continued milk production.

Follicle stimulating hormones (FSH) regulates the development, growth, pubertal maturation and reproductive processes of the human body while Luteinizing hormone (LH) stimulates ovulation, maintains corpus luteum and secretion of progesterone in females(Mahesh, 2012).

Extracts and isolates from almost all parts of *Xylopia aethiopica* plant tend to possess one bioactivity or another that confirms its traditional uses, and have largely shown to be of low toxicity. The survey conducted by Kadiri *et al.*, (2015) revealed that the stem bark of *Xylopia aethiopica* (in combination with other medicinal plants) is used topically as an alcoholic decoction in the treatment of postpartum breast infections. However, results of some of the studies on this plant do not agree with some of its traditional therapeutic uses. Despite many studies on this plant, its effects on sex hormones and lactation in postpartum women have not been given adequate attention, hence the need for this study.

The aim of this study was to investigate the acute effects of xylopic acid on sex hormones and lactation in postpartum female wistar rats.

MATERIALS AND METHOD

Plant materials*:* Dry fruits of *Xylopia aethiopica* were purchased from the New Benin market in Benin City.

Extraction of Xylopic acid: Xylopic acid was extracted using the method described by Kofie *et al.,* (2016). The dried fruits (1.494 kg) were pulverized and soaked in petroleum ether (40-60°C) for 72 hours. The petroleum ether extract was drained and concentrated using rotary evaporator at 60°C. Ethyl acetate was added to the concentrated mass and allowed to stand for 48 hours to allow formation of XA crystals. The concentrate was decanted after the period to separate the deposited crystals from the upper oily mass. The crude xylopic acid formed was purified using recrystallization by dissolving it in ethanol. The resulting solution was filtered and left to stand for 72 hours to recrystallize. The crystals obtained were washed with cold pet ether 40/60 and dried to give xylopic acid as white crystals 0.960g (yield 0.064%).
The structure of XA is as shown below.

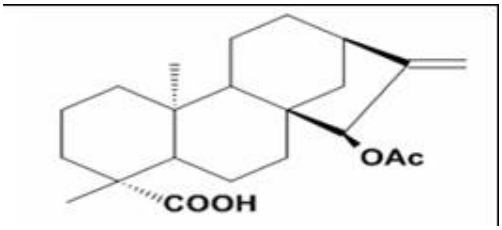

Figure 2: The structure of XA

Molecular weight: 360.487 g mol-1, Molecular formula: C22H32O4
Structure of Xylopic Acid. [15_-acetoxy-(-)-kaur-16-en-19-oic acid] (Kofie *et al.*, 2016)

Experimental Animals: Twenty-five female and 10 male adult Wistar rats were used for this study. These animals were obtained from the Animal House of the Faculty of Pharmacy, University of Benin following the institutional ethical approval. They were kept under standard laboratory conditions. Water and feeds were provided *ad libitum.* The animals, two male rats per five female rats in a cage) were left together for two weeks to allow mating and conception to occur. Fifteen of the female rats confirmed to be pregnant were isolated for the study (test and control).

Experimental Design: The pregnant female rats were randomly assigned to three different experimental groups (n=5 x 3 groups). The first group served as control and was given distilled water orally. The second and third groups were given low dose of 25mg/kg body weights and high dose of 100 mg/kg body weights of Xylopic acid respectively, once daily for seven days. In all the groups, xylopic acid was administered orally immediately after parturition. All animals were allowed free access to food and water throughout the experiment.

Blood Collection: Twenty four hours after the last doses were administered; the animals were anaesthetized with chloroform vapour, before they were sacrificed at about 9am. Whole blood was collected by cardiac puncture from each animal into clean dry sample bottles. The blood in the clean dry sample bottles was allowed to stand for about 15minutes to clot and then was spun in a Westerfuge centrifuge for five minutes. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for the measurements of hormonal profile using enzyme-linked immunosorbent assay (ELISA) (MicroWell™ assay kits, Monobind Inc. Lake forest, CA 92830 USA). Serum levels of Prolactin, progesterone, estrogen, LH, FSH and Testosterone were estimated using ELISA kits as described by the manufacturer. Statistical analysis was performed with GraphPad Prism (version 7.03).

The data were analyzed using one way ANOVA and the results are expressed as mean ± standard error of mean. P value ≤ 0.05 was considered as statistically significant.

RESULTS

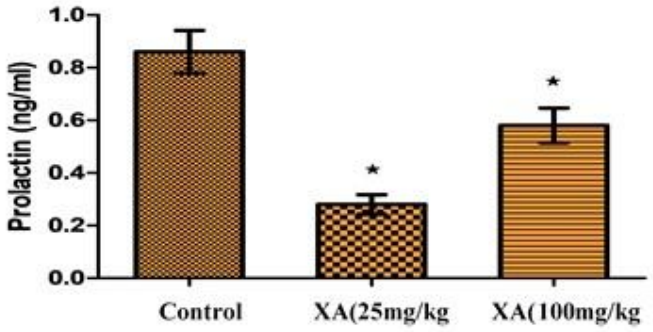

Figure 3: Serum prolactin levels in female rats treated with xylopic acid for seven days postpartum.

There was a significant decrease (P<0.05) when treatment groups were compared to the control group.

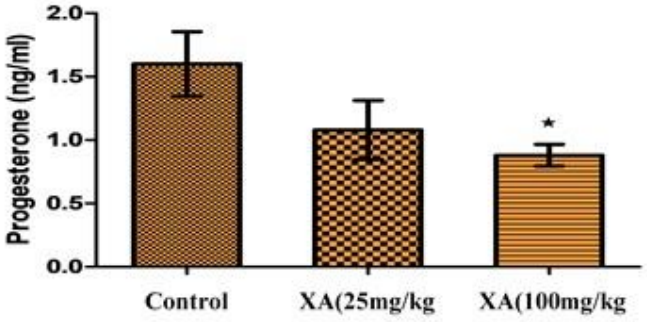

Figure 4 Serum progesterone levels in female rats treated with xylopic acid for seven days postpartum.

There was a significant decrease (P<0.05) when 100 mg/kg BW treatment group was compared to the control group. However, the decrease was not significant (P>0.05) when the low dose 25 mg/kg BW treatment group was compared to the control group.

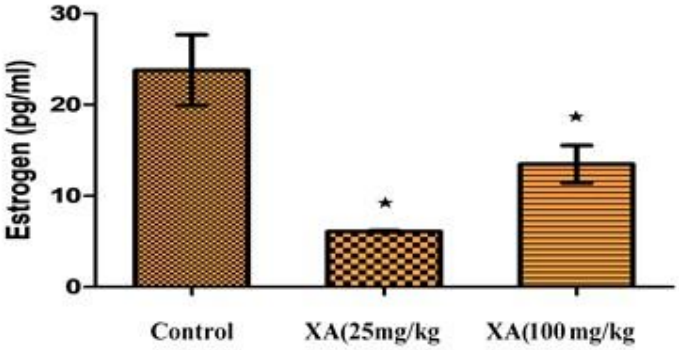

Figure 5: Serum estrogen levels in female rats treated with xylopic acid for seven days postpartum.

There was a significant decrease (P<0.05) when treatment groups were compared to the control group.

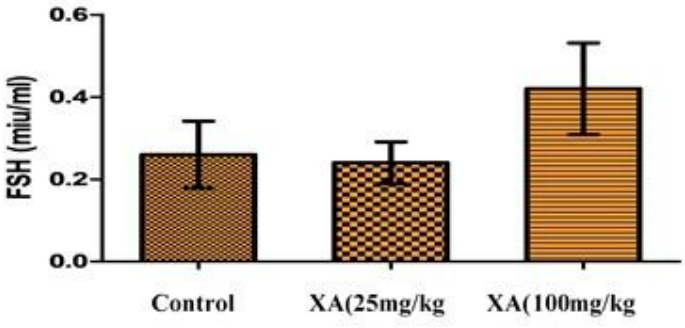

Figure 6: Serum FSH levels in female rats treated with xylopic acid for seven days postpartum.

There was incremental change when 100 mg/kg BW treatment group was compared to the control group. However, the observable change was not significant (P>0.05) for both treatment groups compared to the control group.

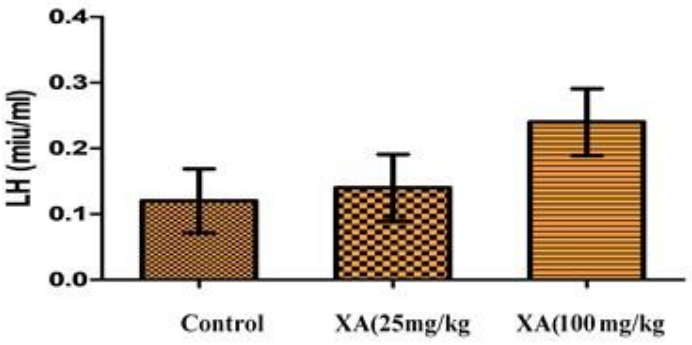

Figure 7: Serum LH levels in female rats treated with xylopic acid for seven days postpartum.

There was incremental change when treatment groups were compared to the control group. However, the increase was not significant (P>0.05) for both treatment groups compared to the control group.

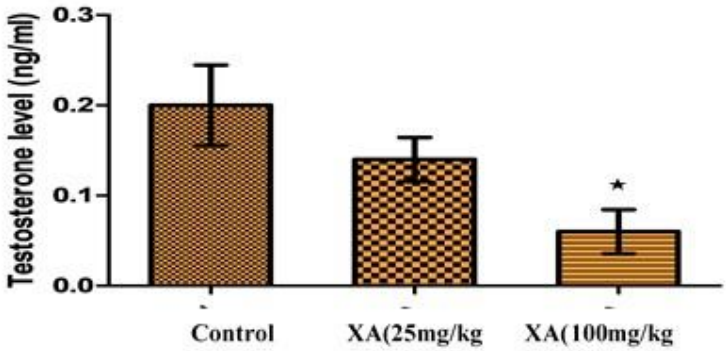

Figure 8: Serum testosterone levels in female rats treated with xylopic acid for seven days postpartum.

There was a significant decrease (P<0.05) when 100 mg/kg treatment group was compared to the control group. However, the decrease was not significant (P>0.05) when the low dose 25 mg/kg treatment group was compared to the control group.

DISCUSSION

Lactation is vital to the survival and wellbeing of newborns as well as to the mothers. Early onset of lactation is so important that it is suggested that about 41 per cent of newborns that die in the first month of life could be saved if breastfed in the first hour of life (Paddock, 2007). The mechanisms controlling lactation are complex and involve preparation of the breast (by progesterone and estrogen) during pregnancy, stimulation of secretion of milk in the immediate postpartum period (mainly by prolactin), ejection of milk from the alveolar cells (by oxytocin), and maintenance of milk production during the period of lactation (by prolactin, growth hormone, etc). Prolactin, Progesterone and estrogen are the main sex hormones involved in lactation. The local effects of estrogen and progesterone in the breast prevent milk secretion during pregnancy. With their withdrawal in the postpartum period, the stimulating effect of the hormone prolactin becomes dominant and milk secretion is initiated and maintained.

The reported dose depended significant reduction (P<0.05) in serum Prolactin concentration in xylopic acid as compared to the control group for 7 days postpartum with observable reduction in serum progesterone high dose 100 mg/kg BW. There was also an incremental effect of XA on serum FSH and LH levels but not significant (P>0.05) probably due to short duration of

treatment. Woode *et al*, (2012) in their work on the effect of XA on sex hormones in male rats showed that duration of treatment had significant incremental effect on serum FSH at 100 mg/kg dose level and they reported significant incremental change for FSH only after 28days of treatment. They also reported a significant dose dependent increase in serum LH after 7 days of treatment as well as after 28days of treatment. The reduction in serum estrogen and progesterone levels strongly suggests that XA suppresses the ovaries, consequently FSH and LH levels tend to rise in response to this suppression. Postpartum increase in the levels of FSH and LH is indicative of inhibition of lactation. Taya and Sasamoto's study (1991) showed that lactation inhibits FSH and LH and that once lactation ceases the levels of these hormones rise within few hours. The results of this study also showed a dose dependent significant decrease ($P<0.05$) in serum testosterone. This result is consistent with the anti-androgenic property of XA reported by Alhassan *et al*., 2013.

Since XA has a reductive effect on Prolactin which is necessary for the onset and maintenance of lactation, it is reasonable to infer that XA has a negative effect on lactation. The mechanism through which this occurred is somehow complex and may be linked with Dopamine which holds a predominant role in the regulation of prolactin secretion (Fitzgeralds *et al.,* 2008). It has been reported that estrogen is essential to the survival of the dopaminergic cells (Redmond *et al.,* 2000) and that ovarian hormones (estrogen and progesterone) even increase dopamine levels in the bodyNestor *et al.,* 2009) yet they (ovarian hormones) enhance prolactin secretion by (1) antagonizing the dopaminergic control (inhibition) of prolactin secretion as a result of decreasing lactotropes responsiveness to dopamine, (Livingstone *et al.,* 1998) (2) recruiting quiescent lactotropes to secrete prolactin and (3) increasing the amount of prolactin secreted by each lactotrope (Close *et al.,* 1997; DeMaria *et al.,* 2000).

CONCLUSION

Since XA significantly reduced serum levels of prolactin, ovarian hormones (estrogen and progesterone), it can be concluded that XA possesses an inhibitory effect on lactation in postpartum female rats through a mechanism that may be associated with the suppression of ovarian hormones which consequently result in prolactin inhibition. Xylopic acid is, therefore, not recommended for post-partum therapy.

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