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Oral administration of aqueous seed extract of *Xylopia aethiopica* to female albino rats, post-partum: effects on the growth and health of the rat pups

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Abstract

The seeds of *Xylopia aethiopica*, commonly referred to as uda, is widely used by women in Nigeria for its purported beneficial effects on uterine cleansing, post-delivery. The effects on the neonates of mothers that use *X. aethiopica* have not been investigated. In the present study, *X. aethiopica* seed extract was orally administered to female rats post-partum (during the breastfeeding period), and the effects on their pups was investigated. Sixteen adult pregnant female albino rats (PFARs), each weighing approximately 180 – 200 grammes, were used for the study. The PFARs after parturition, were randomly assigned into four experimental groups (A, B, C and D) comprising four females (n = 4) in each group. Group A was the untreated control group. Groups B, C and D were treated with 250, 500 and 1000 mg/kg body weight of aqueous *Xylopia aethiopica* seed extract (AXASE) once daily via oral gavage from day zero post-partum until the day their pups were weaned (day 21). The rat pups' litter weights, capillary refill time, normal head posture, eye opening, teeth eruption, fur development and auricular folding were assessed during the 21 day period of lactation period. Results showed that the mean capillary refill time of the pups of all the extract-treated groups were significantly lower ($p < 0.05$) than that of the untreated control group, with the group treated with the lowest dose (250 mg/kg AXASE) having the lowest mean capillary refill time. The mean litter weight of pups from Group B rats (treated with 250 mg/kg AXASE) and D rats (treated with 1000 mg/kg AXASE) was significantly higher than those of pups from Groups A (untreated control) and C rats (treated with 500 mg/kg AXASE). Developmental parameters of albino rat pups (normal head posture, eye opening, teeth eruption, fur development, and auricular folding) were normal, and there were no differences between the groups. No physical anomalies or mortalities were recorded for the pups of all the groups up to the weaning period. The results of this study suggest that *Xylopia aethiopica* seed extract, as used in the study, is safe for pups and helpful in the neonatal growth and development period.

Keywords: *Xylopia aethiopica* seed extracts; Rat pups; Growth; Health; Post-partum; Neonatal growth and development.

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Introduction

The post-partum period, also referred to as the post-natal phase, is a critical time following parturition, during which both mothers (dams) and their neonates undergo significant physiological and emotional adjustments that are vital for their long-term health and well-being (Lopez-Gonzalez and Kopparapu, 2022; O'Dea *et al*., 2023). Effective post-partum care is essential not only for the survival of both mother and neonate, but also for promoting the healthy growth of the newborn (Lopez-Gonzalez and Kopparapu, 2022). This immediate post-partum period is particularly sensitive; complications can arise that may lead to adverse outcomes if not properly handled (Clarke-Deelder *et al*., 2023).

Post-partum complications can manifest in various forms, including excessive bleeding, breastfeeding difficulties, urinary incontinence, mood disorders such as depression or anxiety, fatigue, sleep disturbances and gastrointestinal issues like constipation (Smith *et al*. 2022; Gmelig Meyling *et al*., 2023). One fundamental aspect of recovery during this time involves uterine involution, the process by which the non-gravid uterus returns to its pre-pregnant state after placental expulsion, which typically occurs under the influence of hormones such as prostaglandins (Sutherland *et al*., 2021). The reduction in uterine weight has been associated with decreases in collagen content and alterations in smooth muscle layer thickness (Omar *et al*., 2005). Human mothers often experience discomfort from uterine contractions known as after-pains alongside lochia discharge (Deussen *et al.,* 2020).

In many developing countries, natural remedies are recommended and used to facilitate maternal recovery, and this may lead to passive transfer of their chemical constituents to the newborns/neonates during the lactation phase (Sibeko and Cordeiro, 2021; Kwon *et al*., 2023). One of such remedies is *Xylopia aethiopica* seed, commonly referred to as uda, which is widely used by women in Nigeria for its purported beneficial effects on uterine cleansing, post-delivery (Yin *et al*., 2019).

Xylopia aethiopica is a tropical plant, commonly referred to as Uda, African guinea pepper or Negro pepper, and which produces dark brown pods containing kidney-shaped seeds rich in essential oils such as paradol and terpinene-4-ol along with various nutritional components including proteins and vitamins A1, B1, B2, C & E (Yin *et al*., 2019; Thompson, 2020). The *Xylopia aethiopica* seed has garnered attention within traditional medicine due to its reported diverse pharmacological properties including anti-oxidant activity, anti-inflammatory effects, anti-microbial capabilities, promotion of uterine involution, induction of respiratory health benefits, weight control support, and purported contraceptive properties (Eke *et al*., 2021).

While ergometrine remains a standard treatment option utilized within healthcare systems for inducing uterine involution (Miller and Ansari, 2022) among women who have access to modern medical facilities in developing regions like Nigeria, most women living in rural and semi-urban areas still rely on herbal alternatives like *Xylopia aethiopica* due to its availability, accessibility and the socio-cultural belief in its efficacy. Despite previous research highlighting its role in facilitating maternal recovery during the post-partum uterine involution processes, there exists a notable gap regarding its potential impact on neonates during breastfeeding periods following parturition. In the present study, *X. aethiopica* seed extract was orally administered to female rats post-partum (during the breastfeeding period), and the effects on various parameters of their pups was evaluated.

Materials and Methods

Experimental Animals: A total of 16 adult pregnant female albino rats, each weighing approximately 180 – 200 grammes, were used for this study. The animals were housed in the Animal Care unit of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka. They were kept in stainless steel cages with mesh tops to ensure proper ventilation. The rats were fed a commercially sourced diet and provided with clean drinking water ad libitum. Environmental conditions included an ambient temperature maintained at 25 degrees Celsius and a controlled light cycle consisting of 12 hours of light followed by 12 hours of darkness. All through the study period, the rats and pups were handled humanely following standard ethical procedures in animal care and use.

Plant Collection and Identification: The plant material used for the study, *Xylopia aethiopica* seeds (Figure 1) were collected from Nsukka Local Government Area. Identification and authentication were done by Mr. Isaac Ossai, curator/taxonomist at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Nigeria. A voucher specimen (InterCEDD/205Xylopia aethiopica) was deposited at the Centre's Herbarium for reference.

Plant Extract Preparation: The aqueous extract of the *Xylopia aethiopica* seeds was prepared using the decoction method as described by Njoku *et al*., (2023). The plant material was sundried and ground into a fine powder. A measured amount (25g) of this powder was then mixed with 100 ml of water, and the mixture was boiled. After boiling, the mixture was sieved to obtain the decoction extract.

Experimental Design: The sixteen pregnant female albino rats were randomly assigned into four experimental groups (Groups A, B, C and D) made up of four females in each group. After delivering their offspring (pups), Group A was the untreated control group. Groups B, C and D were treated with 250, 500 and 1000 mg/kg body weight of aqueous *Xylopia aethiopica* seed extract (AXASE) once daily via oral gavage from day zero post-partum until the day their pups were weaned (day 21). During the lactation period following birth up until weaning, the following parameters were evaluated: litter weight of the pups, timing of eye opening, capillary refill time, auricular unfolding stages, teeth eruption patterns, alongside head posture alignment assessments as well as overall pup death rates.

Figure 1. *Xylopia aethiopica* pods (above) and the seeds used for the study (below it).

Statistical analysis: Quantitative data obtained during the study were subjected to one-way analysis of variance (ANOVA), and variant means were separated using the least significant difference (LSD) post-hoc test. Probability values less than 0.05 were considered significant. All the analysis was carried out using the SPSS 16.0 statistical package of SPSS Inc. USA. (Iranloye, 2011; Steele and Torrie, 1980). Summary results were expressed as mean ± standard error of mean (SEM) in tables.

Results

The mean capillary refill time of the pups of all the extract treated groups were significantly lower ($p < 0.05$) than that on the untreated control group, with the group treated with the lowest dose (250 mg/kg AXASE) having the lowest mean capillary refill time and the one treated with the highest dose (1000 mg/kg AXASE) having the highest capillary refill time, among the treated groups (Table 1). The litter weight of pups of Group B rats (treated with 250 mg/kg AXASE) and that of Group D rats (treated with 1000 mg/kg AXASE) was significantly higher than those of Groups A (untreated control) and C (treated with 500 mg/kg AXASE) [Table 1].

The results of the effect of maternal administration of *X. aethiopica* seed extract on some neonatal developmental parameters of albino rat pups are presented in Table 2. Normal head posture, eye opening, teeth eruption, fur development, and auricular folding were normal for all the groups, with no differences. No physical anomalies were observed in any group and no mortalities were recorded all through the 21 day lactation period.

Table 1: Effect of aqueous decoction extract of *X. aethiopica* (ADEXA) administered to female rats post-partum on the mean capillary refill time and litter weights of their albino rat pups.

Groups	Capillary refill time (s)	Litter weight (g)
Group A (Untreated Control)	2.3 ± 0.04 [a]	60.1 ± 3.7 [a]
Group B (250 mg/kg ADEXA)	1.5 ± 0.01 [b]	79.4 ± 2.3 [b]
Group C (500 mg/kg ADEXA)	2.0 ± 0.04 [c]	61.9 ± 2.2 [a]
Group D (1000 mg/kg ADEXA)	2.1 ± 0.03 [d]	74.4 ± 2.4 [b]

Results are presented as means ± SE; Different alphabetical superscripts on the same column shows significant difference at $p < 0.05$

Table 2: Effects of aqueous decoction extract of *X. aethiopica* (ADEXA) administered to female rats post-partum, on some developmental milestones of their albino rat pups.

Groups	Parameters						
	HP	**EO**	**TE**	**FD**	**PA**	**DR**	**AF**
Group A (Untreated Control)	Normal	Normal	Normal	Normal	None	None	Normal
Group B (250 mg/kg ADEXA)	Normal	Normal	Normal	Normal	None	None	Normal
Group C (500 mg/kg ADEXA)	Normal	Normal	Normal	Normal	None	None	Normal
Group D (1000 mg/kg ADEXA)	Normal	Normal	Normal	Normal	None	None	Normal

HP = Head posture, EO = eye opening, TE = teeth eruption, FD = Fur development, PA = physical anomaly, DR = death rate, AF = auricular folding.

Discussion

The results of this study indicated a dose-dependent response to *Xylopia aethiopica* aqueous seed extract in albino rat pups. Notably, capillary refill time was reduced across all doses compared to the control group, suggesting that the extract may influence peripheral circulation and tissue perfusion in the developing rats. Improved capillary refill time could imply enhanced blood flow, which may contribute to nutrient delivery to tissues, potentially supporting the observed body weight differences. Interestingly, while both the highest (1000 mg/kg) and lowest dose (250 mg/kg) led to significantly higher litter weight compared to the untreated control; the medium dose did not yield the same effect. This non-linear dose response suggests a complex interaction between dosage and physiological effects, where certain concentrations may reach a threshold beyond which the expected effect does not occur, possibly due to metabolic adaptation mechanisms at the medium dose level. The findings of this study align with previous research suggesting that *Xylopia aethiopica* seed extract possesses bioactive compounds with potential beneficial effects. The positive effects observed could be attributed to the presence of flavonoids and alkaloids in the seed extract, which are known to have antioxidant and anti-inflammatory properties (Brown and Green, 2018). This could explain the observed improvements in growth and health markers, such as capillary refill time (Johnson and Lee, 2019; Smith *et al*., 2021). These antioxidants may help reduce oxidative stress, thereby supporting healthier growth and development. Additionally, the anti-inflammatory properties of *Xylopia aethiopica* seed extract may mitigate inflammatory responses that could otherwise impede growth (White *et al*., 2022). These results suggest that *Xylopia aethiopica* seed extract could play a role in enhancing developmental health by counteracting oxidative damage and inflammation.

Developmentally, rat pups from groups receiving the *Xylopia aethiopica* extract exhibited normal head posture, timely eye opening, teeth eruption, fur development, and auricular folding. These findings, alongside the absence of physical anomalies or mortality, suggest that the extract is well-tolerated and does not adversely affect normal development up to the weaning period. The lack of observed toxic effects is encouraging and supports the potential safe use of *Xylopia aethiopica* during developmental stages, at least within the dosing range used. Our study concurs with the findings of Smith *et al*. (2021) by demonstrating tangible benefits in a developmental context. For instance, Smith *et al*. (2021) reported improvements in immune function and growth parameters in animal models treated with *Xylopia aethiopica* seed extract. Our study extends this understanding by focusing on the effects during early developmental stages, which is critical for establishing safe and effective dosages for potential therapeutic use. Overall, this research contributes to understanding the impact of *Xylopia aethiopica* on young mammals, indicating potential benefits without adverse developmental effects. However, further studies are recommended to explore the long-term impact and underlying mechanisms of the dose-dependent variations observed in body weights and other physiological responses.

Conclusion: Oral administration of aqueous decoction extract of Xylopia aethiopica to female rats post-partum led to significantly lower capillary refill time in the pups of the treated groups and some differences in the litter weights of the pups based on the doses. No physical developmental anomalies and mortality were associated with the administration. Further research is recommended on the seed extracts of *X. aethiopica*.

Conflict of interest

The authors declare no conflict of interest.

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