

## The effect of Negro Pepper (*Xylopi aethiopica* fruit) extract on Prostaglandin E<sub>2</sub> and connexin 43 levels in the early and mid-gestation period of pregnant Wistar rat

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**ABSTRACT**

Excessive prostaglandin E2 production and aberrant increase in connexion 43 level during pregnancy had been reported to lead to uterine contraction and consequently pregnancy loss. This study investigated the effect of negro pepper (NP) extract on prostaglandin E2 (PGE2) and connexion 43 (Cx 43) levels in pregnant Wistar rats. 40 virgin female Wistar rats weighing between 150g to 180g were purchased from the animal house of the Department of Anatomy, University of Benin. The dry fruits (NP) were ground to powder form and soaked with n-hexane using maceration method for 24 hrs and the resultant mixture was filtered with filter paper to obtain the filtrate which was evaporated to dryness using a rotary evaporator to obtain the oil. The female rats were mated with male rats to achieve pregnancy. The pregnant rats were grouped into two phases: Germinal phase and Embryonic Phase and each phase was divided into four groups (A, B, C and D) of n=5 rats each. The first three groups (A, B and C) in each phase served as test groups and were administered orally 0.25, 0.5 and 1ml/Kg b.wt of the hexane extract of XA fruit respectively while group D served as control in each phase of gestation. 24 hours after the last administration, the rats were sacrificed using chloroforms anaesthesia, and their uteri were excised and used for the biochemical analysis. In conclusion, NP extract significantly decreased PGE2 level across all doses while Cx43 level increased significantly at 0.5 and 1ml/kg b.wt of extract compared to control in the Germinal phase. However, PGE2 and Cx43 levels significantly increased at 0.5ml/kg and 1mg/kg b.wt. in the embryonic phase, showing that the extract exhibits a phase-dependent biochemical alteration which point towards the disrupted endometrial receptivity and accelerated myometrial contractility. Consequently, the consumption of hexane extract of NP should be strictly avoided due to its ability to trigger uterine contractions, which may result in pregnancy loss.

**Keywords:** negro pepper; prostaglandin E2; connexin 43; pregnancy; n- Hexane extract.

## INTRODUCTION

*Xylopia aethiopica* (XA) is a tropical evergreen aromatic plant which belongs to the Annonaceae family (OBIRI & OSAFO, 2015). XA grows in the savanna regions of Africa, particularly Ghana, Nigeria, Cameroon, Ethiopia, and Senegal (YIN et al., 2019). The fruit of XA is commonly known by different names such as guinea pepper, Ethiopian pepper, black pepper etc. Traditionally the fruit is used in the treatment of inflammatory disorders, malaria, (OSO & OLADIJI, 2019), amenorrhea and in the induction of placenta discharge (ABOLAJI; ADEBAYO; ODESANMI, 2007). The fruit has also been used as spice in the preparation of local soup (pepper soup) meant for pregnant women to alleviate feverish conditions and stimulate appetite sometimes.

Phytochemicals reported most abundant in the essential oil of XA fruit include:  $\beta$ -pinene,  $\alpha$ -pinene, caryophyllene, bisabolene, naphthalene, phellandrine, terpinene-4-ol (alcohol), 1,8-cineole (oxide), kaur-16-ene etc (FETSE; KOFIE; ADOSRAKU, 2016).

Connexins are gap junction proteins allows cell to cell communication and allows free flow of ions and small molecules between cells (LAIRD, 1996). In rats and rabbits Connexin 43 expression is induced locally in the endometrium in response to implantation of the blastocysts (GRUMMER & WINTERHAGER, 1998) to the stroma compartment and suppressed by maternal progesterone level during pregnancy. In the rabbit and humans, the receptiveness of the endometrium is characterized by a lack of cell-cell communication channels (GRUMMER & WINTERHAGER, 1998).

Connexin 43 expression is low throughout pregnancy but increases only during term and preterm labor in the myometrium (ORSINO; TAYLOR; LYE, 1996). Prostaglandins (PGS) are group of physiologically active lipid compounds (eicosanoids) (RICCIOTI & FITZGERALD, 2011). Derived enzymatically from arachidonic acid by cyclooxygenase (COX), also called prostaglandin endoperoxide synthase (PTGS) (JOSEPH et al., 2013). Present in maternal blood, urine, and amniotic fluid (BAKKER; PIERCE; MYERS, 2017). Levels change dynamically during pregnancy: Early pregnancy: low levels  $\rightarrow$  maintain stability, prevent premature labor. Late pregnancy: gradual increase in PGE<sub>2</sub> and PGF<sub>2</sub> $\alpha$  near term (NIRINGIYUMUKIZA; CAI; XIANG, 2018). Preterm labor: excessive prostaglandin production results uterine contractions, premature cervical changes, preterm birth (THOMAS et al., 2014).

However, the expression of connexin 43 in the myometrium is highly dependent on

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the intracellular signaling of prostaglandin E2 especially prostaglandin F<sub>2α</sub> enhancing myometrial contractility of the uterus during labor (SPAREY et al., 1999). The aim of this article study is to determine the effect of n-Hexane extract of *Xylopi aethiopica* fruit on prostaglandin E2 and Connexin 43 level in pregnant Wistar rats.

## MATERIALS AND METHODS

### *Plant Material*

Identification and authentication; Dried fruits of *Xylopi aethiopica* was purchased from New Benin Market, Edo State, Nigeria and authenticated by comparing it with an already existing sample in the herbarium at the Department of Plant Biology and Biotechnology, University of Benin, and was assigned a voucher number UBH:X348 which was deposited at the herbarium for future reference.

### *Preparation of Extract*

Pulverized fruit (1kg) was extracted with 2.5litres of n-hexane using maceration method for 24hrs. The mixture was filtered and the filtrate rotary evaporated to obtain 40 g dark-brown oil extract. Stored in refrigerator until when needed for use (AZEKHUMEN & EBOMOYI, 2024).

### *Ethical Approval*

Ethical approval was sorted t the Research Ethics Committee of the College of Medical Sciences, University of Benin with Ethical approval number CMS/REC/2025/820 was assigned.

### *Establishment of the LD50 of the Hexane extract of XA (HEXA) fruit*

The LD50 of the HEXA was determined in two phases according to Lorke' method of 1983. In phase one nine (9) rats were used' The rats were randomised into 3 groups (A, B and C) of n=3 rats each. Each group were administered 10, 100 and 1000mg/kg of the HEXA respectively and observed for 24 hours for signs and symptoms of toxicity. In phase two three (3) rats were used. The rats were randomised into 3 groups (A, B and C) and each group was administered 1600, 2900 and 5000mg/kg of the HEXA respectively and observed for signs

and symptoms of toxicity 24hour and then for two weeks for delay sign of toxicity such as death.

#### *Dosing of the HEXA Fruit oil*

To determine the dose of the oil to be administered, the density of the oil was first determined by weighing 1ml of the oil in three consecutive measurements (g) and the average of the three taken. The density of the oil was then calculated to be 0.940g/ml equivalent to 940mg/ml. The dose of the oil to be administered was calculated based on the body weight of the rats as follows; - weight (kg) x Dosage ordered (mg/kg) divided by concentration of the oil (mg/ ml). For a rat weighing 175g equivalent to 0.175kg, the dose of the oil to be administered at an ordered dose of 1000mg/kg will therefore be calculated as; - 0.175 kg x 1000mg/kg divided by 940mg/ml = 0.186ml.

#### *Experimental Animals*

Female Wistar rats (150–180g) from Anatomy Department Animal House, University of Benin. Acclimatization: 2 weeks, 24–27 °C, 12 h light/dark cycle, feed and water ad libitum.

#### *Mating of Animals*

The female rats were paired with male rats in the ratio (2:1) in breeding enclosure, chosen to minimize male aggressive behaviors.

#### *Pregnancy Detection methods*

Vaginal smear examination for sperm, Observation of vaginal plug. The day of sperm detection was designated as Gestation Day 0 (AZEKHUMEN & EBOMOYI, 2024).

#### *Experimental Design*

The pregnant rats were divided into 2 phases: Germinal phase (gestation day 0–7) and Embryonic phase (gestation day 8–14). Each phase was divided into 4 groups (A, B, C and D) with group A serving as control in each phase.

Group A: 0.25 XA oil extract orally

Group B: 0.5ml/kg X.A oil extract orally

Group C: 1.0 ml/kg X.A oil extract orally

Group D: control

7 days via orogastric tube according to (AZEKHUMEN & EBOMOYI, 2024)

### *Sample Collection*

24 hrs after last doses, rats were sacrificed via chloroform anaesthesia and Uteri samples collected and homogenated in phosphate buffer solution, centrifuged at 3000 rpm for 10 minutes. Supernatant collected into plain tubes for biochemical analysis of prostaglandin E2 and CX 43 levels according to the method described by Nair et al. (2011).

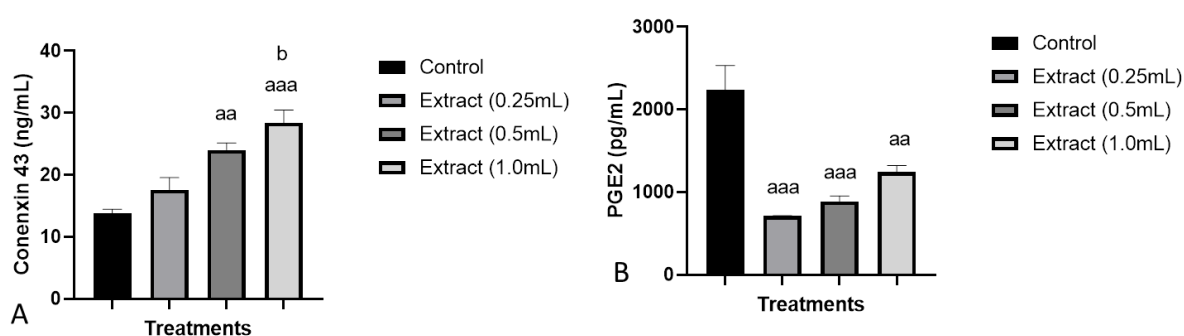
### *Statistical Analysis*

Data were analysed using graph pad prism software version 10.6.1. Results were expressed as Mean  $\pm$  SEM. One-way ANOVA was used for group comparison followed by Bonferroni post hoc test.  $P < 0.01$  was considered statistically significant compared to control.

## **RESULTS**

Figure 1A illustrates the extract's impact on Connexin 43 and PGE2 expression, while Figure 1B demonstrates extract' influence on Prostaglandin E2 concentrations, highlighting the extract's potential modulatory role on key molecular markers during early pregnancy.

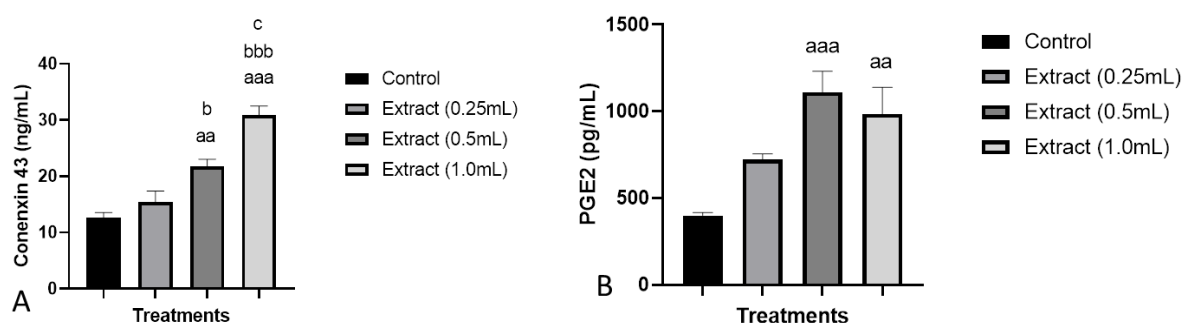
There was a significant increase in Connexin 43 (Cx43) levels at 0.5 mL/kg and 1.0 mL/kg doses of African Pepper Extract compared to the control group, while no significant increase was observed at the 0.25 mL/kg dose in the germinal phase. Statistical analysis revealed that  $aap < 0.001$  and  $aap < 0.01$  when compared with the control and  $bp < 0.05$  when the higher doses were compared to the 0.25 mL/kg group. Conversely, Prostaglandin E2 (PGE2) levels showed a consistent decrease across all extract doses relative to control, with significance levels of  $aap < 0.001$  and  $aap < 0.01$ .



**Figure 1.** The effects of African Pepper Extract on pregnant Wistar rats during the germinal phase were evaluated by examining changes in both Connexin 43 and Prostaglandin E2 levels.

Figure 2A illustrates the extract's influence on Connexin 43 expression, while figure 2B highlights its impact on Prostaglandin E2 concentrations, suggesting a potential modulatory role of the extract on key molecular markers involved in embryonic development.

There was a significant increase in Connexin 43 (Cx43) levels across all treatment groups compared to the control, with statistical markers indicating  $aaap < 0.001$  and  $aap < 0.01$  versus control, and  $bbbp < 0.001$  and  $bp < 0.05$  versus the 0.25 mL/kg extract group. Similarly, Prostaglandin E2 (PGE2) levels significantly increased at 0.5 mL/kg and 1.0 mL/kg doses of the extract compared to control ( $aaap < 0.001$ ;  $aap < 0.01$ ), while no significant increase was observed at the 0.25 mL/kg dose. Further comparisons showed  $cp < 0.05$  when the 1.0 mL/kg group was compared to the 0.5 mL/kg group.



**Figure 2.** The effects of African Pepper Extract during the embryonic phase of pregnancy in Wistar rats were assessed by measuring changes in Connexin 43 and Prostaglandin E2 levels.

## DISCUSSION

In mammals, the implantation of the embryo into the uterine tissue is strictly restricted to the receptive phase of the endometrium and for effective implantation to occur there must be proper differentiation of the receptive endometrium. Connexin 43 (Cx 43) and Prostaglandin E2 (PGE2) play a crucial role in the endometrial receptivity of the developing embryo (GRUMMER & WINTERHANGER, 1998; NIRINGIYUMUKIZA et al., 2018).

During early pregnancy PGE2 play an important role in female fertility including; ovulation, enhancement of sperm binding to oocyte (fertilization), reduce viscosity of the extracellular matrix (blastocyst transport to the uterus), early embryo implantation (NIRINGIYUMUKIZA et al., 2018) and in the formation of new blood vessels from already existing vasculature in a process known as angiogenesis (XIN et al., 2021). In decidualization (functional permeability of the endometrial wall by the developing embryo for implantation by transforming stromal cells into secretory cells necessary for implantation) Prostaglandin E2 acts primarily through specific receptors (EP1 and EP4) to upregulate progesterone receptors through the PGE2-PKA-CREB1 signaling pathway and promote growth of primary cilia on the endometrial cell preparing the uterine lining both physically and chemically for the reception of the developing embryo (LEE et al., 2025).

Cx43 function to promote gap junction formation between myometrial cells allowing cell to cell communication and exchange of ionic substances including hormones to be exchanged between cells via their cytoplasm (LEYBAERT et al., 2017). Cx43 stimulate the differentiation of the stromal cells to produce VEGF which is necessary for the growth of blood vessels that were produced due to PGE2 signaling further promoting transport of the blastocyst to the uterus enhancing the receptivity of the maternal uterus to the developing embryo for implantation. Failure of PGE2 signaling to occur prevent Cx43 expression leading to implantation failure and subsequently pregnancy loss (SPAREY et al., 1999).

Grummer and Winterhanger (1998) reported that, in mammals such as rats, rodents and humans, endometrial receptiveness of the embryo is characterized by a lack of cell to cell communication channels.

In this study, there was a significant decrease in PGE2 levels across the graded doses compared to control at the germinal phase. This is in agreement with the work done by Kim et al. (2013) who reported a similar decrease in PGE2 level using isolated uterine strip from a rat in an in vitro study. The decrease in PGE2 level observed in this study, also agrees with

the work of An et al. (2013) who reported a similar reduction in inflammation due to a decrease in TNF- $\alpha$  and PGE2 levels in rats pre-administered chamaecyparis obtuse (C.Obtuse) essential oil after the induction of inflammation with lipopolysaccharide in an *in vivo* study.

The significant decrease in PGE2 level observed in this study, may be as a result of the sabinene content of XA fruit extract.

Sabinene has been reported to inhibit the activities of the enzyme cyclooxygenase which is necessary for the conversion of arachidonic acid into prostaglandins (KIM et al., 2013). A significant decrease in PGE2 level in the germinal phase in this study, is an indication that XA oil extract disrupted endometrial receptivity of the developing embryo due to failure of PGE2 signaling and may have as well inhibited implantation of the embryo to the uterine wall. Cx43 level was increased significantly at 0.5ml/kg and 1ml/kg in the germinal phase though there was no significant increase at 0.25ml/kg b.wt of extract compared to control in this study. This contradicts the report by Grummer and Winterhanger (1998) that Cx 43 expression is suppressed in the endometrial stromal compartment by progesterone during gestation in rats.

During early pregnancy Cx43 level remains low but gradually rise to peak at term or labor (GRUMMER et al., 1994). Cx 43 level is induced locally in the endometrium by the implanting blastocyst (WINTERHANGER et al., 1993) but its expression becomes suppressed in the endometrium by the upregulated progesterone receptors (maternal progesterone) which was induced by prostaglandin signaling resulting in a lack of cell to cell communication during gestation (GRUMMER & WINTERHANGER, 1998; LEE et al., 2025).

Excessive or significant increase in Cx43 level in the germinal phase or receptive phase endometrium in this study disrupts the normal down regulation of gap junctions required for successful embryo implantation, indicating that there was a mistimed cellular differentiation and altered fetomaternal communication leading to implantation failure.

In the embryonic phase there was a dose dependent increase in both Cx43 and PGE2 levels at the 0.5ml/kg and 1ml/kg b.wt of the extract compared to control though there were no significant increases at 0.25ml/kg b.wt of the extract. This finding is in support of the work done by Sparey et al. (1999) who reported that Cx43 expression is strictly dependent on prostaglandin E2 signaling. The increase in PGE2 level observed in this study is in agreement with the work of Furtado et al. (2002) who reported a similar increase in PGE2 level in an *in*

*vivo* study with rats which he suggested may be as a result of the sabinene content in the fruit extract. The increase in part due to the fact that some of the fatty acids present in the HEXA fruit may have been channeled into the prostaglandin synthetic pathway. The collaborated increase in PGE2 and Cx43 levels at the embryonic phase of the rat pregnancy shows that this phase may be sensitive phase during which the XA oil extract induced a synchronized myometrial smooth muscle contraction orchestrated by the updistorting the quiet state of the endometrium which is required for successful pregnancy. Significant increase in Cx43 in the embryonic phase observed in this study, may also result to abnormal proliferation of the trophoblastic cells leading to early pregnancy loss.

## CONCLUSION

Consumption of n-hexane extract of X.A fruit significantly decreased PGE2 level across all groups and significantly increased Cx 43 level at the germinal phase but collaboratively increased both PGE2 and CX 43 levels significantly at 0.5ml/kg and 1.0ml/kg in the embryonic phase of pregnant Wistar rats indicating that the extract may pose a risk to pregnancy at both phases of pregnancy which could be more effective at the embryonic phase of gestation.

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