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Gravid Uterine Responses in Wistar Rats administered *Pterocarpus santalinoides* at Late Pregnancy

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ABSTRACT

There are increasingly significant safety concerns regarding the use of medicinal plants with regard to maternal and fetal health. This is particularly due to the established capacity of some bioactive compounds like phytoestrogens, saponins, and others to influence reproductive hormone levels, enhance contraction of the uterus, and jeopardize the integrity of the myometrium. The effect of ethanol leaf extract of *Pterocarpus santalinoides* (ELEPS) on the gravid uterus of primigravid Wistar rats during late pregnancy, gestational day 15 – 21, was investigated in this study. Pregnant rats were divided into 5 groups: A, B, C, D, and E, and administered 10ml/kg distilled water, 50 µg/kg misoprostol, 400 mg/kg ELEPS, 600 mg/kg ELEPS, and 800 mg/kg ELEPS, respectively. Results showed dose-dependent adverse effects, including significant weight reduction and complete fetal loss across ELEPS-treated groups C, D, and E. Fetuses exhibited intrauterine growth restriction at lower doses, while the highest dose induced early abortion with uterine involution and hemorrhage. Histological analysis revealed increased endometrial folding, inflammatory cell infiltration, and hyperemia. Immunohistochemistry demonstrated elevated expression of uterine oxytocin receptors (OTR) and cyclooxygenase-2 (COX-2), indicating enhanced contractile and inflammatory pathways. These findings suggest that *Pterocarpus santalinoides* possesses potent uterotonic and fetotoxic properties, which may be mediated through hormonal disruption, estrogen potentiation, and prostaglandin synthesis, and poses significant risks in pregnancy. The findings from this research suggest that intake of *Pterocarpus santalinoides* during late gestation is unsafe.

Keywords: *Pterocarpus santalinoides,* late pregnancy, oxytocin receptors, Wistar rats

INTRODUCTION

Pregnancy is a complex physiological process that involves embryogenesis, fetal organogenesis, and development of the placenta [1], characterized by a relatively quiescent uterine environment devoid of coordinated contractions, necessary for optimal fetal development [2]. It is marked by intricate adaptations that guarantee the survival and development of the growing fetus through balanced exchange of nutrients, elimination of wastes, and protection from external stimuli [3, 4].

Late pregnancy, a phase known as the fetal phase, is characterized by highly increased fetal growth as it is usually a period of increase in fetal size and weight [5]. At this phase, the fetus undergoes organ maturation, accumulates fat storage reserves, strengthens neural connections, and prepares for extrauterine life [6,7].

In the rural communities of sub-Saharan Africa, it is often assumed that the foetus is completely immune to external influences at the late stage of pregnancy. This is worrisome as the use of medicinal plants during gestation has raised quite a lot of concerns [8]. Using medicinal plants to treat ailments during late pregnancy could be detrimental as bioactive compounds in herbs can cross the placenta, and affect foetal programming, the epigenetic process that influences long-term cell growth, structure, and function, thereby altering cell growth and structure, leading to cell, tissue, and organ modifications [9]. The eventual adverse outcomes include altered organogenesis, intrauterine growth restriction, or even fetal loss through mechanisms like apoptosis induction or vascular disruption.

Pterocarpus santalinoides, a plant within the family Fabaceae, is seen throughout the tropical regions, including West Africa [10]. It is commonly called red

sandal wood plant in English [11] and identified with local communities in Nigeria as Gyadar kurmi in Hausa; nturukpa (Igbo); gbengbe (Yoruba) [12]. The plant, which is edible and used as a food ingredient [13] can grow to be a big tree with low straggling branches or as small as a shrub, within grazing reach of livestock under semi-extensive or extensive method of livestock production. In Nigeria, for instance, *Pterocarpus santalinoides* is used as food or medicine. The tender leaves are used as a vegetable in soup making, while the stem bark is used in making pepper soup [10]. Studies indicate that the plant exhibits potent antibacterial properties [14], therefore, its use in the treatment of numerous diseases and inflammatory conditions [13]. The ethno-medical use of leaves of *Pterocarpus santalinoides* in the treatment of diarrhoea and other gastrointestinal disorders has been scientifically reported [15]. In southeast Nigeria, leaves of *P. santalinoides* are ethnomedicinally used against diabetic syndrome and for the management of pyrexia [16]. The leaves are also used for the treatment of skin diseases [17]. Its stem-bark and leaf extracts are said to possess anti-enteropooling, antimalarial, anti-abortive, and antibacterial properties [18, 19]. The use of the plant, Pterocarpus santalinoides, in the management of rheumatism, diarrhea, dysentery, cough, asthma, elephantiasis, cold, and others has been reported [20, 21]. The use of the leaves in treating skin diseases such as eczema, candidiasis, and acne has also been reported [22].

Pterocarpus santalinoides exhibits activities against markers of oxidative stress [23] and hepatoprotective properties [24], supporting its use as a regular source of herbal medicine. Analysis on the phytoconstituents of *Pterocarpus santalinoides* suggests the presence of complex bioactive compounds like flavonoids, saponins, tannins, alkaloids, phytoestrogens [10, 11].

Consistently, experimental studies have shown that various medicinal plants possess embryotoxic, teratogenic, or abortifacient properties that function to disrupt hormonal activities, induce uterotonic contractions through calcium channel activation or prostaglandin release, or trigger oxidative stress and inflammation in the utero-placental unit [25]. This raises significant safety concerns, particularly due to the established capacity of phytoestrogens, saponins, and other bioactive compounds in medicinal plants to influence levels of endogenous reproductive hormones, enhance myometrial contractility, jeopardize the integrity of the decidua, and negatively impact maternal and foetal health [13]**.** Explicit reports [26, 27] show that phytoestrogens (genistein, daidzein, isoflavone) at feto-maternal interphase bind to estrogen receptors, and act as hormone disruptors negatively affecting pregnancy. Saponins, tannins, and steroids in plants are uterotonic in action and have been associated with abortifacient activities in pregnant subjects [28, 29, 30]. Earlier reports [31] opine that tannins may elicit a uterotonic effect by stimulating calcium availability for uterine muscle contraction. The activities of the phytochemicals pose direct threats to maternal-fetal health, potentially causing preterm labor, low birth weight, or congenital anomalies, which may go undetected in rural settings where there are limited resources for prenatal monitoring.

In the current study, the effect of *Pterocarpus santalinoides* on the pregnant uterus of Wistar rats was assessed to determine its safety at late pregnancy and also serve to enlighten commercial/household livestock farmers, as well as women in sub-Saharan Africa, on the effects of *Pterocarpus santalinoides* on the pregnant uterus, especially at the late stage.

MATERIALS AND METHODS

Research animals and care

Sexually mature (adult) nulligravid normocycling female Wistar rats, weighing between 140g and 223g, were obtained from the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, and used for this study. The animals were maintained under standard laboratory conditions with a 12–hour light–dark cycle at a room temperature of 25 ± 2 °C and fed with standard pelleted grower's feed (Ladokun Farms Ltd., Nigeria). Water was given *ad libitum*.

Ethical consideration

This research was conducted using the guidelines of the EU Directive 2010/63/EU. Accordingly, the study was evaluated and approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU/ CVM/REC/202123). The research animals were managed following international guidelines for laboratory animal care.

Estrous cycle monitoring and mating of research animals

The estrous cycle of the rats was monitored using daily vaginal cytology procedures [32]. Rats showing regular reproductive cycling of 4 days observed for two consecutive cycles were selected at the proestrus stage of the cycle and paired for mating with proven fertile males at a 2:1 ratio [33]. Evidence of successful mating was seen in female rats exhibiting the presence of vaginal plugs or sperm cells in the vaginal smear the following morning. The day of the observation was considered Gestational Day 1 of pregnancy (GD 1) as described by other authors [34, 35].

Experimental groups

The experiment was carried out during the late pregnancy phase, designated as GD 15 – GD 21. (Note that pregnancy in rats runs for a period of 21

gestational days (GD), thus, dividing the gestational length into 7 gestational days per phase. Thus, the first phase, the early pregnancy phase, is represented by GD 1– GD 7, the second phase, the mid pregnancy phase, is represented by GD 8 – GD 14, while the third phase, the late pregnancy phase, is represented by GD 15 – GD 21.

The primigravida rats at GD 14 were randomly sorted into five (5) groups – Group A, Group B, Group C, Group D, and Group E, each made up of 6 pregnant Wistar rats.

Group A (negative control/untreated group) -10ml/kg distilled water only,

Group B (the positive control group) - standard drug, Misoprostol® tablets at 50 µg/kg

Group C - 400 mg/kg ethanol leaf extract of *Pterocarpus santalinoides*.

Group D - 600 mg/kg ethanol leaf extract of *Pterocarpus santalinoides*.

Group E - 800 mg/kg ethanol leaf extract of *Pterocarpus santalinoides*.

Treatments as indicated were administered once daily, orally using gastric gavage starting from GD 15 to GD 21at 09:00 hours.

Experimental plant: *Pterocarpus santalinoides*:

Fresh leaves of the experimental plant, *Pterocarpus santalinoides*, were collected during the dry season from behind Veterinary Post Mortem Laboratory, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Following routine proper botanical identification at the Department of Plant Health and Biotechnology, using the standard botanical identification protocol earlier described [36], a voucher number MOUAU-PSL-2023-11 was allocated to the plant. The leaves were washed with clean water, air-dried, and then pulverized into fine powder in preparation for plant extraction.

Plant extraction

The pulverized plant leaves (500 g) were soaked in 80% ethanol (Sigma Aldrich Germany) at room temperature in a reagent amber bottle in preparation for cold maceration. The solution was vigorously shaken at intervals for a period of 72 hours to ensure complete extraction. Thereafter, the extract was filtered through a Whatman No 1 filter paper into a measuring beaker. The filtrate was then dried at a temperature of 45 °C under reduced pressure.

The percentage yield (w/v) of the extract was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of material extracted} \times 100}{\text{Weight of plant material} \quad 1}$$

The extraction yield was calculated to be 12.4% w/w based on the dry weight of the plant material.

Standard drug, misoprostol®

The standard drug used was Misoprostol® (Taj Pharma© India). Misoprostol® is a synthetic analogue of prostaglandin E_1 used off-label during pregnancy for the induction of labour, medical abortion, cervical ripening, and in handling conditions of postpartum bleeding [37]. The drug was used at a dose of 50 µg/kg [38] and given orally using gastric gavage.

Experimental procedure

Daily live weight of experimental animals

Daily, the rats in all the experimental groups were individually weighed using an electronic weighing balance (Saitorius® England, model TE 15025). The weights were obtained to the nearest 0.05 accuracy and recorded in grammes.

Hysterectomy

Twenty-four (24) hours after the final treatment, on gestational day 22, three (3) rats randomly selected from each group were sacrificed by cervical dislocation under light ether anesthesia. The uterus of each of the animals was carefully exteriorized following midline laparotomy, and observed for pregnancy status [39]. The number of implantation and resorption sites, retained viable fetuses, and overall gross uterine morphology were observed and properly recorded, and observations were compared among groups.

Pregnancy losses among the groups were evaluated by computing the percentage survival ratio and post-implantation loss using the description of previous studies [39].

% Survival ratio = (number of live fetus /number of live + dead fetus) ×100

Post-implantation loss = (number of implantations - number of live fetuses/number of implantations) ×100.

Thereafter, the fundus of the uterus was excised for tissue preparation for histomorphology and immunohistochemistry.

Tissue preparation for histology and immunohistochemistry

The placental tissues, fetuses, fat, and connective tissue were carefully excised from the uterus. The tissue was immediately fixed in 10% neutral-buffered formalin for 24 hours and then passed through an increasing concentration of ethanol baths until dehydrated in 100% ethanol twice. They were then embedded in paraffin wax and processed for routine histology. The surface of the paraffin wax blocks was cooled with an ice block, and 5µm sections were cut using a rotary microtome (Leica RM2125RT, Germany) for histological studies and immunohistochemistry.

For histology studies, the sections were stained with hematoxylin and eosin (H&E) following standard protocols [40] and examinations were conducted using a light microscope. Parameters such as endometrial folding, epithelial proliferation, endometrial gland morphology, Pigmentation, hyperemia, and inflammatory cells were assessed.

Semi-quantitative scoring system: (-) absent, (+) mild, (++) moderate, and (+++) marked, was used to grade histological changes [41].

Immunohistochemistry was conducted using the standard procedure [42]. Cut sections were placed on microscope glass slides and baked at 37°C overnight. The sections were dewaxed in three changes of xylene, rehydrated in ethanol, and the endogenous hydrogen peroxide activity quenched in 3% H_2O_2 solution in methanol for 30 minutes. Subsequently, the sections were rehydrated in descending grades of ethanol. The slides were then washed with 1xPBS and subjected to antigen retrieval, cooled, washed twice with 19 PBS, and blocked at room temperature with blocking serum for 1 hour. Primary antibody incubation (using individual primary antibodies for oxytocin receptor and cyclooxygenase 2, each at 1:200) was performed overnight at 4°C. Following the primary antibody incubation, the slides were washed thrice in 1xPBS. Biotinylated secondary anti-rabbit antibody (1:300) was then used against the species of primary antibody for 1 hour at room temperature. Thereafter, the slides were washed in PBS, and streptavidin-labelled horseradish peroxidase-conjugated antibody was applied to the slides for 30 minutes at room temperature. Following this, the slides were developed using 0.02% H_2O_2, 0.075% 3,3-diaminobenzidine (3,3- DAB) solution in a 19 PBS staining kit. Counterstaining was done, the sections dehydrated, cleared, and mounted with a mounting medium. Negative controls for immunohistochemistry studies were produced following omission of primary antibodies and incorporating Rabbit immunoglobulin G (IgG) (1:200) at the same concentration as the primary antibodies. Photomicrographs of the slides were viewed and assessed using semi-quantitative [43], and graded as (-) absent, (±) very weak, (+) mild, (++) moderate, and (+++) intense.

Data analysis

Data from the study were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) version 23. Results obtained were presented as mean ± standard error of the mean (SEM). Varying means were separated using the least significant difference (LSD) post-hoc test. Values were considered statistically significant at $p<0.05$.

RESULTS

Effects of *Pterocarpus santalinoides* on the daily average live weight of Wistar rats at late pregnancy

The daily average live weights of the pregnant rats in each group are presented in Figure 1. *Pterocarpus santalinoides* treatment had an undulating effect on the live weight of the rats in the group treated with 400mg/kg ELEPS, which is the low dose of the extract in this study. There was a decline in weight at day 4 and day 7 of the experiment. A steady decline in average live weight was observed in groups D and E treated with 600 mg/kg ELEPS and 800 mg/kg ELEPS, respectively, which are higher doses of the extract.

Effects of administration of ethanol leaf extract of *Pterocarpus santalinoides* on the pregnancy status of Wistar rats at late pregnancy

The groups treated with the ELEPS – 400 mg/kg, 600 mg/kg, and 800 mg/kg, C, D, and E, respectively, all had complete foetal losses (Table 1). Rats in group A showed no abortion. All pups were born normal and alive at term. The rats in Group B, treated with misoprostol 50 µg/kg, delivered stillbirth pups. Observation from the study showed that group C rats, treated with 400 mg/kg ELEPS, had grossly undersized pups (evidence of intrauterine growth restriction), and more notably, the size of the placentae tissues from group C was comparable to those from the pups found in group B treated with misoprostol 50 µg/kg. Group D gravid uterus showed small-sized non-viable fetuses, suggesting evidence of intra-uterine growth restriction. The Uterus from Group E rats, administered the high dose of the extract, 800 mg/kg ELEPS, showed an apparently involuted uterus with haemorrhagic bands at the implantation sites, which were considered evidence of pregnancy, and an early abortion.

Effects of *Pterocarpus santalinoides* on the gravid uterus of Wistar rats at late pregnancy

Photomicrograph of the histological sections of the gravid uteri of Wistar rats in all the groups is shown in Figure 2. Group A rats, administered 10 ml/kg distilled water (Figure 2), exhibited marked endometrial folding, with multifocal areas of epithelial proliferation and apoptosis. There was a mild influx of inflammatory cells and hyperemia. Hypertrophy of the myometrial layers was also observed. The endometrial glands showed epithelial proliferation.

Assessment of the histological sections of the gravid uterus from Group B showed marked endometrial folding, multifocal areas of epithelial proliferation, and apoptosis. Multifocal infiltration of inflammatory cells (leucocytes) into the endometrium was observed with some hyperemic areas. Endometrial glands with proliferative epithelium, in addition to hypertrophy of the myometrial layers, were also seen.

Group C treated with 600 mg/kg ELEPS exhibited marked endometrial folding with multifocal areas of epithelial proliferation and apoptosis. Mild to moderate multifocal infiltration of inflammatory cells into the endometrium with some hyperemic areas. Hypertrophy of the myometrial layers was also observed.

Table 1: Effects of *Pterocarpus santalinoides* on the pregnancy status of Wistar rats at late pregnancy

Treatment Groups	Average Number of Implantation Sites	Average Number of Foetuses Lost	Average Number of Live Fetuses Born	Percentage Survivability (%)	Post-implantation loss (%)	Remark
A (Distilled water 10 ml/kg)	8.66±1.45	0.00± 0.00[b]	8.66±1.45[a]	100.00	0.00	No abortion
B(misoprostol® 50 µg/kg)	10.00±1.52	10.00±1.52[a]	0.00±0.00[b]	0.00	100.00	Total foetal loss, stillbirth
C (ELEPS 400 mg/kg)	10.66±2.33	10.66±2.33[a]	0.00±0.00[b]	0.00	100.00	Abortion, IUGR in all the retained foetuses, and total foetal loss
D (ELEPS 600 mg/kg)	11.66±1.45	11.66±1.45[a]	0.00±0.00[b]	0.00	100.00	Abortion, IUGR in all the retained foetuses, and total foetal loss
E (ELEPS 800 mg/kg)	9.66±0.88	9.66±0.88[a]	0.00±0.00[b]	0.00	100.00	Complete abortion and early uterine involution

Values are expressed as Mean ± S.E.M. Means with different superscript letters along columns differ significantly at $p<0.05$. ELEPS – ethanol leaf extract of *Pterocarpus santalinoides*, IUGR – Intrauterine growth restriction

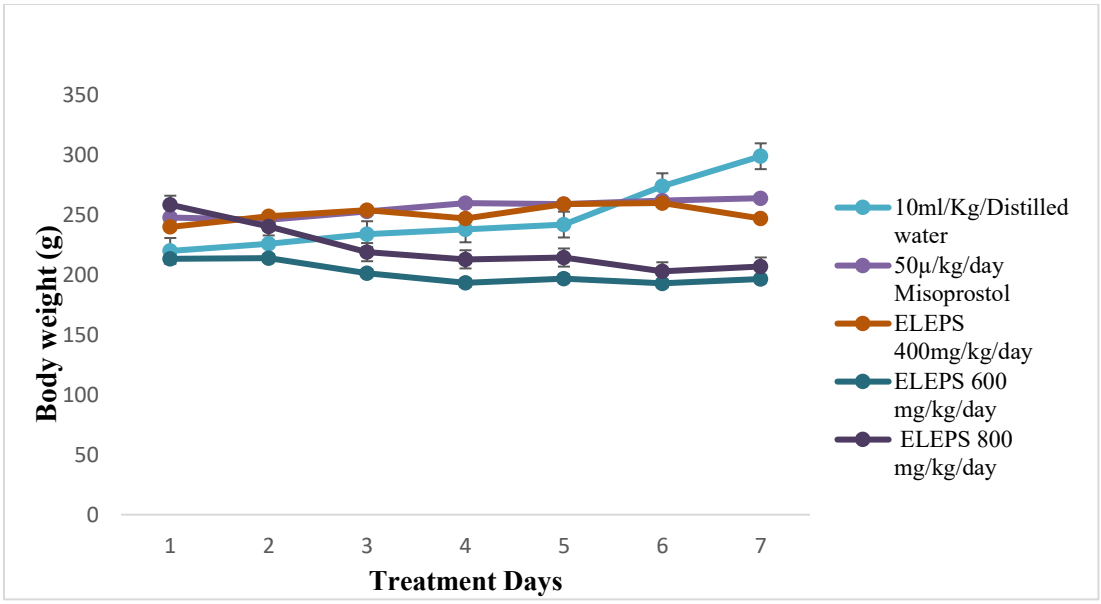

Figure 1: Effects of Ethanol leaf extract of *Pterocarpus santalinoides* (ELEPS) on live weight of Wistar rats at late pregnancy.

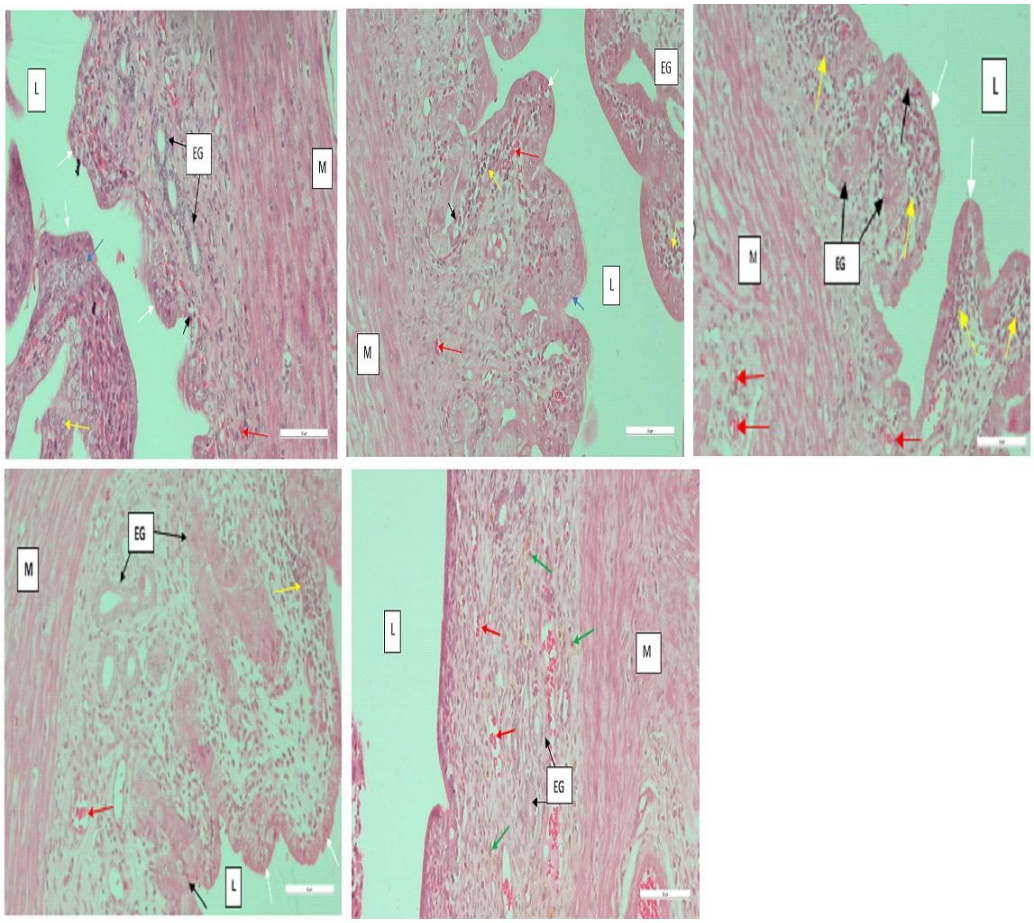

Figure 2: Photomicrographs of gravid uteri of Wistar rats in late pregnancy (H/E X400). Arrows indicate areas of protein expression. ELEPS - Ethanol Leaf Extract of *Pterocarpus santalinoides*. Black arrow – Cell apoptosis, Blue arrow - Epithelial proliferation, Green arrow – Hemosiderin, EG - Endometrial glands, L- Lumen, M – Myometrium, Red arrow – Hyperemia, White arrow - Endometrial folding, Yellow arrow - Inflammatory cells. **A**-Group A (Distilled water 10 ml/kg) rats, **B**- Group B (Misoprostol 50 µg/kg) rats, **C**- Group C (ELEPS 400 mg/kg) rats, **D** - Group D (ELEPS 600 mg/kg) rats, **E** - Group E (ELEPS 800 mg/kg) rats.

Marked endometrial folding, with multifocal areas of epithelial proliferation and apoptosis, characterized the uterine histology in group D rats treated with 800 mg/kg ELEPS. Endometrial glands also exhibited epithelial proliferation in the group. There was increased multifocal infiltration of inflammatory cells into the endometrium with some areas of hyperemia. Hypertrophy of the myometrial layers was also observed.

Gravid uterine sections from Group E rats administered the high dose of the extract (800 mg/kg ELEPS) showed uterine endometrium lined by cuboidal to low columnar epithelial cells. Endometrial glands were more numerous when compared to the other groups, and glands showed epithelial proliferation. The endometrium showed multifocal deposition of golden-brown pigments interpreted to be hemosiderin. Increased hyperemia was observed in this group when compared to other groups A, B, C, and D. There was mild leucocytic infiltration, and hypertrophy of the myometrial layers was seen.

All the gravid uterine tissues obtained from the groups (Table 2) showed significant folding of the endometrial lining except in group E administered the high dose of the extract (800 mg/kg). Slight leucocytic infiltration was observed in group A treated with distilled water at 10ml/kg, but more in B, C, and D, administered misoprostol 50 µg/kg, 400 mg/kg ELEPS, and 600 mg/kg ELEPS, respectively. Leucocytic infiltration was very low in group E treated with 800 mg/kg ELEPS, where it was obvious that there had been an early abortion, and the uterus was apparently undergoing involution. Hyperemia was seen in all the groups, though it was marked in group E, treated with 800 mg/kg ELEPS. Golden brown pigments interpreted to be hemosiderin were also observed in the endometrium of only group E tissues of rats treated with 800 mg/kg ELEPS.

Effects of *Pterocarpus santalinoides* on gravid uterine tissue expression of oxytocin receptor (OTR) and cyclooxygenase 2 (COX-2) at late pregnancy in Wistar rats.

Images A, B, C, D, and E (Figure 3) representing groups A, B, C, D, and E, respectively, showed organized aggregated expression of oxytocin receptors (OTR) in the myometrium and endometrium of the gravid uteri of all the groups. The lowest OTR expression was seen in the control group (A), while groups B, C, and D, treated with misoprostol® 50 µg/kg, 400 mg/kg ELEPS, and 600 mg/kg ELEPS, showed the highest levels of expression. The level of immunostaining was graded as Group A (+), Group B (++), Group C (++), Group D (++), and Group E (+).

Where, – indicates no positive staining in any cell, +/- indicates very weak staining in ~1% of the cells, + indicates pale staining, ++ indicates mild staining, and +++ indicates intense staining.

Immunohistochemical sections of gravid uteri of the treatment groups (Figure 4) showing the profile of uterine tissue expression of the protein, cyclooxygenase 2 (COX-2), revealed no protein expression in the control group (A) administered 10 ml/kg distilled water. However, intense expression of the protein was observed in both the myometrium and the endometrium in groups B, C, D, and E treated with 50 µg/kg Misoprostol, 400 mg/kg ELEPS, 600mg/kg ELEPS, and 800mg/kg ELEPS, respectively. The level of immunostaining was graded as Group A (-/+), Group B (+++), Group C (+++), Group D (+++), and Group E (+++); where, – indicates no positive staining in any cell, +/- indicates very weak staining in ~1% of the cells, + indicates pale staining, ++ indicates mild staining, and +++ indicates intense staining.

DISCUSSION

Ethanol leaf extract of *Pterocarpus santalinoides* (ELEPS) in this study exerted marked contractile and fetotoxic effects at late gestation in the Wistar rats, resulting in complete fetal losses in all the ELEPS-treated groups. Sites of abortion and retained fetuses with characteristics of delayed growth and development (indicators of intrauterine growth restriction) seen in rats administered low and medium doses of the extract of *Pterocarpus santalinoides* are evidence of teratogenicity. Intrauterine growth restriction is known to disrupt fetal programming, which leads to altered cell, tissue, and organ growth and structure [9]. These effects from *Pterocarpus santalinoides* could be attributed to its phytoconstituents, including saponins, isoflavonoids, and phytoestrogens, [10, 11,] which may have disrupted vascular flow to the developing foetus following uterine contractions in the treated pregnant dams [38, 44]. Specifically, former reports [26,27] show that phytoestrogens bind to estrogen receptors, disrupting normal hormone balance at feto-maternal interphase, intercepting pregnancy. Isoflavonoids have been linked to placental insufficiency and embryotoxicity, while saponins and steroids in plants provoke uterine hypercontractility and have been associated with abortifacient activities in pregnant subjects [27-29]. Early manifestations of abortion, evidenced by localized pinpoint hemorrhagic implantation sites and observed uterine involution, following administration of the high dose of *Pterocarpus santalinoides* leaf extract, are indications of complete pregnancy interceptory activities at that dose.

Table 2: Summary of gravid uterine histological findings across treatment groups in Wistar rats treated with *Pterocarpus santalinoides* during late pregnancy

GROUP	Endometrial folding	Epithelial proliferation	Endometrial glands	Pigmentation	Hyperemia	Inflammatory cells
A (distilled water 10ml/kg)	+++	+++	++	-	++	++
B (misoprostol® 50µg/kg)	+++	+++	++	-	++	+++
C (400mg/kg ELEPS)	+++	+++	++	-	+	+++
D (600mg/kg ELEPS)	+++	+++	++	-	+	+++
E (800mg/kg ELEPS)	-	+	+++	++	+++	+

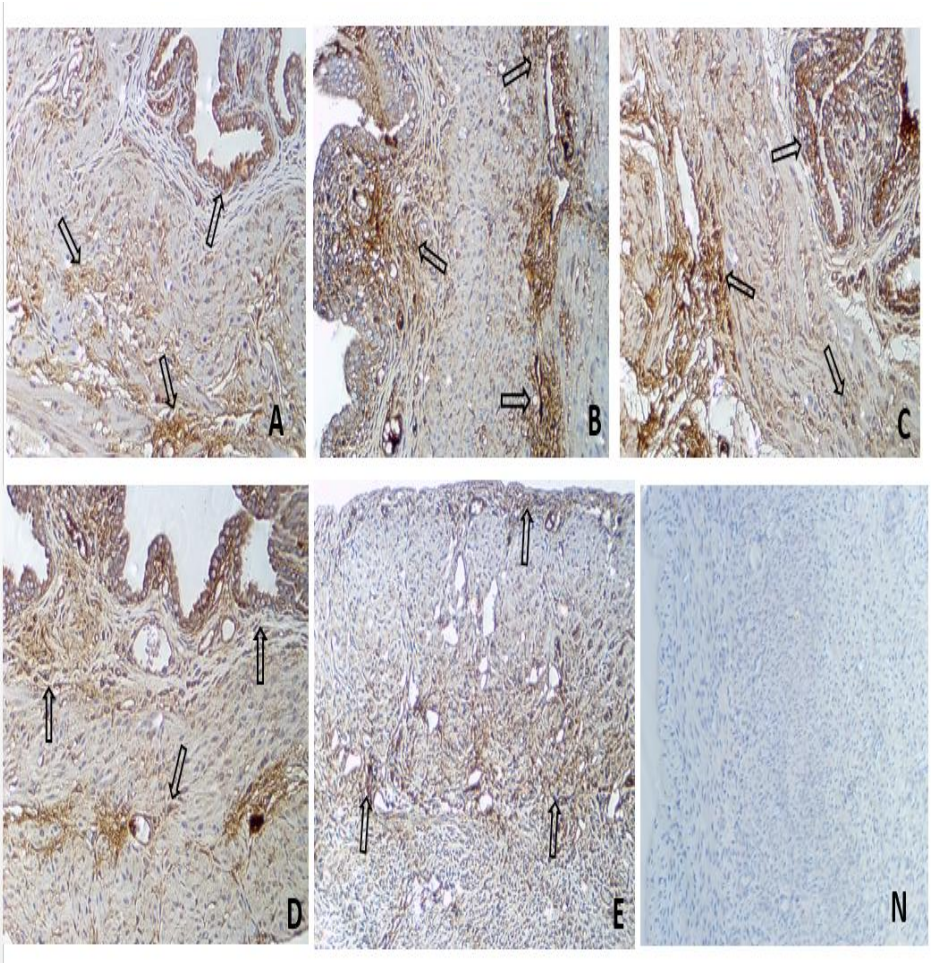

Figure 3: Photomicrograph showing the effect of *Pterocarpus santalinoides* on gravid uterine tissue expression of oxytocin receptors (OTR) in Wistar rats at late pregnancy. **A**-Group A (Distilled water 10 ml/kg) rats, **B**- Group B (Misoprostol 50 µg/kg) rats, **C**- Group C (ELEPS 400 mg/kg) rats, **D** - Group D (ELEPS 600 mg/kg) rats, **E** - Group E (ELEPS 800 mg/kg) rats. Arrows indicate some areas of protein expression. ELEPS - Ethanol Leaf Extract of *Pterocarpus santalinoides*. N - Negative control for oxytocin receptor

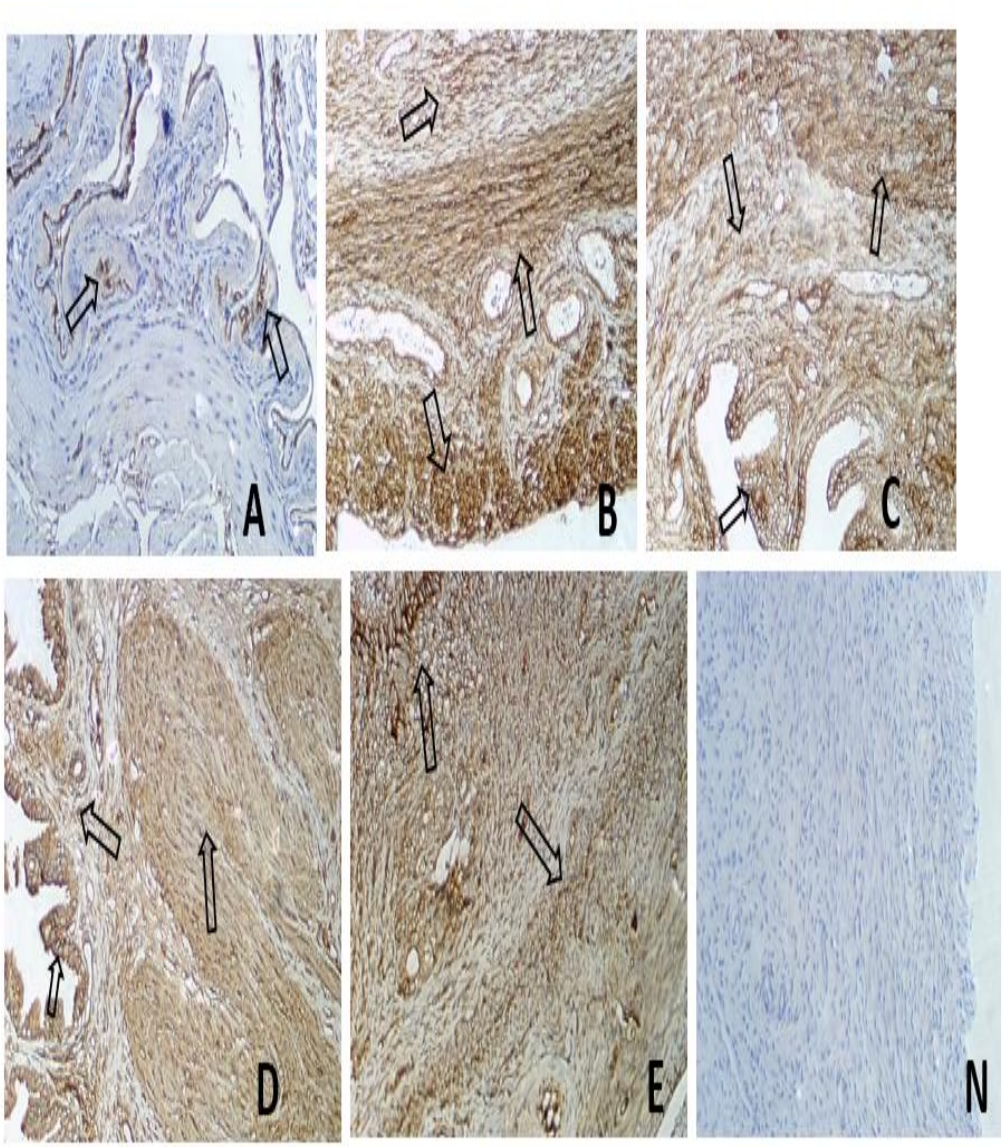

Figure 4: Photomicrograph showing the effect of *Pterocarpus santalinoides* on gravid uterine tissue expression of cyclooxygenase 2 (COX-2) in Wistar rats at late pregnancy. **A**-Group A (10ml/kg Distilled water) rats, **B**- Group B (50 µg/kg Misoprostol) rats, **C**- Group C (400 mg/kg ELEPS) rats, **D** - Group D (600 mg/kg ELEPS) rats, **E** - Group E (ELEPS 800 mg/kg) rats. Arrows indicate some areas of protein expression. ELEPS - Ethanol Leaf Extract of *Pterocarpus santalinoides*. N - Negative control for cyclooxygenase 2

The observed haemorrhagic sites may have been formed from the breakdown of the decidua, which is known to play a critical role in the establishment and continuity of pregnancy [45]. The decidua is the heterogeneous multicellular maternal tissue, essential for endocrine and immunological activities, very closely associated with the fetoplacental unit, and critical to successful pregnancy outcome [46]. During decidualization and placentation, there is massive tissue remodeling and notable alterations in the structure and function of the deposited extracellular matrix (ECM), regulated by matrix metalloproteinases (MMPs) [47-49]. Therefore, abnormal expression of MMPs leads to impaired decidua, resulting in decreased maternal tolerance to uteroplacental disorders, apoptosis of invasive trophoblast cells during pregnancy, reduced uterine perfusion pressure, miscarriages, and other adverse pregnancy outcomes [45, 47].

Studies have shown that some phytochemicals, like phytoestrogens, flavonoids, and terpenoids, exert modulatory effects on MMPs and disrupt maternal response to hormonal signaling [26, 50]. Phytoestrogens bind to estrogen receptors at feto-maternal interphase [27], resulting in negative pregnancy outcomes. Previous studies report the presence of phytoestrogens, flavonoids, and terpenoids in *Pterocarpus santalinoides* [10, 11]. It can therefore be inferred that these phytochemicals in *Pterocarpus santalinoides* may have been responsible for the pregnancy

interceptory activities in the pregnant rats. Uterine involution, marked by myometrial contractions and endometrial atrophy, mimics postpartum regression, driven by prostaglandin surges and progesterone withdrawal. This could be due to the elevated estrogen levels resulting from the activities of phytoestrogens in *Pterocarpus santalinoides* binding to estrogen receptors at feto-maternal interphase in the pregnant rats. The activity of ethanol leaf extract of *Pterocarpus santalinoides* on the pregnant uterus, at this stage of gestation, underscores the risks associated with the use of herbal medicine in late pregnancy.

The steady rise in live weight observed in the groups administered distilled water and misoprostol, respectively, pointed to an uninterrupted progression in pregnancy in these groups. The rats administered a low dose of the *Pterocarpus santalinoides* extract exhibited fluctuating weights, which may be due to the interference of the low dose of the extract with fetal growth and development during the course of pregnancy. Higher doses of *Pterocarpus santalinoides* extract affected a consistent reduction in live weight, demonstrating an increased pregnancy interceptory activity in a dose-dependent manner.

Histological findings showed uterine tissues with folding of the endometrial lining in all the rats except those treated with a high dose of the extract, which possibly had early abortion and were apparently undergoing uterine involution at the time of sample collection. In addition, the presence of hemosiderin in the same rats could be attributed to the breakdown of extravasated red blood cells resulting from post abortion hemorrhages [51].

The findings from the expression of contraction-associated proteins - oxytocin receptor (OTR) and Cyclooxygenase-2 (COX-2) in the gravid uterine tissues of the rats suggest that treatment of pregnant rats with *Pterocarpus santalinoides* may have increased the expression and the activities of contraction-associated proteins in a dose-dependent manner.

Increased expression of oxytocin receptors (OTR) in the endometrium and the myometrium in the rats administered low and medium doses of the extract in this study may have led to intense uterine contraction, disrupting the uterine milieu and resulting in the fetal losses recorded in the study.

OTR, the only receptor for oxytocin through which it mediates its effect, belongs to the rhodopsin-type class I G-protein coupled receptor (GPCR) superfamily [52, 53]. Studies report that OTR activates the mitogen-activated protein kinase (MAPK) and the Rho kinase pathways [53, 54]. Whereas Rho-associated protein kinases induce cell migration, cell cycle control, and cell contractility, MAPK causes an upregulation of cyclooxygenase-2 (COX-2) [54, 55]. The same authors also suggest that activation of both OTR and MAPK elevates cytosolic phospholipase A2 (cPLA2) levels, leading to an increase in arachidonic acid. Increased arachidonic acid subsequently raises prostaglandin production via cyclooxygenase-2 (COX-2) protein activity. OTR also activates receptor-coupled channels, which leads to membrane depolarization and the entry of extracellular Ca2+ into the cells [53]. This triggers various responses in the cells and further increases smooth muscle contraction. Upregulation of OTR concentration in myometrial cells is mediated by estrogen [56], and phytoestrogens known to increase estrogen levels and activities are present in *Pterocarpus santalinoides* [10,11]. Thus, the administration of *Pterocarpus santalinoides* may have increased estrogen levels, leading to upregulation of OTR concentration in the uteri of the treated subjects. Pale OTR staining observed from the administration of the high dose of the extract may be a result of complete abortion and subsequent uterine involution that had occurred earlier in the course of the study. The complete abortion, comparable to parturition and the preceding uterine involution, may have contributed to the rapid decline in OTR expression, corroborating earlier reports [57].

Cyclooxygenase-2 is a rate-limiting enzyme in prostaglandin production, induced in response to pro-inflammatory stimuli because significant upregulation of cyclooxygenase 2 has been associated with prostaglandin synthesis and inflammatory response [58]. These reports validate the findings from the present study in rats administered misoprostol and the low and medium doses of the extract, which exhibited increased leucocytic infiltration in the histological study of the gravid uterine tissues. Increased leucocytic infiltration is one of the signs of an inflammatory response [59].

The expression of COX-2 is also regulated by sex hormones, with estrogen specifically acting as its upregulator [60]. The marked expression of COX-2 in the extract-treated rats in this study further substantiates the estrogen-potentiating activity of *Pterocarpus santalinoides*, through its phytoestrogen content. Phytoestrogens bind to estrogen receptors at feto-maternal interphase in the pregnant rats, [27] leading to increased estrogen levels, and subsequent marked expression of COX-2. The upregulated expression of OTR and COX-2 points to the uterine contractile activity of the phytoconstituents of *Pterocarpus santalinoides,* indicating its disruptive activities on pregnancy even at the late stage.

CONCLUSION

The administration of ethanol leaf extract of *Pterocarpus santalinoides* during late pregnancy induced intrauterine growth restriction, fetal losses, early uterine involution, and upregulated expression of OTR and COX-2 in a dose-dependent manner. The findings from this study point to the toxic activities of bioactive compounds in *Pterocarpus santalinoides,* highlighting the risks of intake of some medicinal plants in late pregnancy.

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REFERENCES

1. Nwachukwu CU. Review article: Nutritional effects on fetal development during gestation in ruminants. Niger. J. Anim. Prod. 2020;(2):245 – 256
2. Arthur P, Taggart MJ, Zielnik B, Wong S, Mitchell BF. Relationship between gene expression and function of uterotonic systems in the rat during gestation, uterine activation, and both term and preterm labour. J Physiol 2008; 586(24):6063-76.
3. Remien K, Majmundar SH. Physiology, Fetal Circulation. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026. Available at: https://www.ncbi.nlm.nih.gov/books/NBK539710/
4. Vornic I, Buciu V, Furau CG, Gaje PN, Ceausu RA, Dumitru CS, *et al.* Oxidative stress and placental pathogenesis: a contemporary overview of potential biomarkers and emerging therapeutics. Int J Mol Sci. 2024;25(22):12195. doi:10.3390/ijms252212195.
5. Kenyon PK, Stafford C, Jenkinson S, Morris D. The body composition and metabolic status of twin-and triplet-bearing ewes and their fetuses in late pregnancy. Livest Sci 2007;107(2):103-112.
6. Symonds M, Mostyn A, Pearce S, Budge H, Stephenson T. Endocrine and nutritional regulation of fetal adipose tissue development. J Endocrinol. 2003;179(3): 293-299. doi:10.1677/joe.0.1790293
7. Clinck B, Allison C, Patino F. The transition to life outside the womb: a review of neonatal physiology, Anaesth Intensive Care Med. 2025; 26(11): 719-729. ISSN 1472-0299, doi:10.1016/j.mpaic.2025.10.001
8. El Hajj M, Holst L. Herbal Medicine Use During Pregnancy: A review of the literature with a special focus on Sub-Saharan Africa. Front Pharmacol. 2020; 11:866. doi: 10.3389/fphar.2020.00866.
9. Sinclair KD, Rutherford KMD, Wallace JM, Brameld JM, Stöger R, Alberio R, *et al.* Epigenetics and developmental programming of welfare and production traits in farm animals. Reprod Fertil Dev. 2016;28(10):1443-1478. doi: 10.1071/RD16102.
10. Nwokorie CC, Nwachukwu NC, Ezeanokete CC, Ike CC. The Phytochemical and antimicrobial analysis of *Pterocarpus santalinoides*. Asian J Sci Technol. 2015;6(05):1411-1418.
11. Ayo-Enwerem MC, Ahaotu EO, Nwogu CM, Esukpa M. Hematology and serum biochemistry of starter broilers fed diets containing red sandalwood (*Pterocarpus Santolinoides*) leaf meal. Direct Res. J. Vet. Med. Anim. Sci. 2017;2(4):110-114.
12. Igoli JO, Igwue IC, Igoli NP. Traditional medicinal practices among the Igede people of Nigeria. J Herbs Spices Med Plants 2003;10(4):1-10.
13. Ay´ena AC, Anani K, Dosseh K, Agbonon A, Gbeassor G. Comparative study of antimicrobial, anti-inflammatory, and antioxidant activities of different parts from *Pterocarpus santalinoides* L'Her. Ex. DC (Fabaceae). Hindawi Evid Based Complement Alternat Med 2021;8938534:1-7.
14. Adeniyi SA, Ehiagbonare JE, Nwangwu SCO. Nutritional evaluation of some staple leafy vegetables in Southern Nigeria. Int. J. Agric. Food Sci. 2021;2(2):37-43.
15. Okpo SO, Ching FP, Ekeleme IC. Evaluation of the anti-diarrhoeal activity of the aqueous extract from leaves of Pterocarpus santalinoides. Res J Pharm Biol Chem Sci. 2011;2(3):590-597.
16. Anowi CF, Umeokoli BO, Onyegbule AF, Okonkwo CCI. Analgesic, phytochemical, and acute toxicity evaluation of the methanol extract of the leaves of *Pterocarpus santalinoides*- family Fabaceae. Int J Pharm Sci Res 2012; 3(7):2018-2023.
17. Igwenyi IO, Elekwa AE. Phytochemical analysis and determination of the vitamin contents of *Geranium robertianum*, IOSR J Dent Med Sci 2014;13(6):44-47.
18. Nworu CS, Akah PA, Nwachukwu JO, Asogwa C. Antidiarrheal activity of *Pterocarpus santalinoides* L'Herit ex DC

leaf extract. J Complement Integr Med 2009;6(1):1-8.
19. Eze SO, Cornelius C, Okereke HC. Phytochemical and antimicrobial analysis of the stem bark of *Pterocarpus santalinoides* (Nturukpa). Asian J Nat Appl Sci, 2012;1(3):26-32.
20. Okwu DE, Ekeke T. Phytochemical screening and medicinal composition of chewing sticks in South Eastern Nigeria. Glob J Pure Appl Sci 2003;9:235-238.
21. Igoli JO. Traditional medicine practice amongst the Igede people of Nigeria, part 11, Int J Biotechnol Allied Sci 2005;4(4):408-412.
22. Adetunji JA. Reviewing Pterocarpus species and their distribution. Afr J Tradit Complement Altern Med 2007;4(2):1-8.
23. Enemali MO, Udedi SC, Ubaoji KI, Haruna GS, Augustine E. Modulatory effect of leaf extracts of *Pterocarpus santalinoides* Hook f. (red sandal wood) on acetaminophen-induced hepatotoxicity in albino rats. Sci Technol J. 2019; 4(2):366 – 371.
24. Ihedioha TE, Asuzu IU, Anaga AO, Ihedioha JI. Hepatoprotective and antioxidant activities of *Pterocarpus santalinoides* methanol leaf extract. Afr J Pharm Pharmacol 2019;13(18):359-373.
25. Ani N, Odirichukwu E, Ekere S, Raheem K, Onu J. *In vivo* effects of ethanol leaf extract of *Pterocarpus santalinoides* on early pregnancy in Wistar rats: a dose-dependent analysis of embryotoxic activities. J Basic Appl Zool. 2026;87:10 doi:10.1186/s41936-025-00538-2
26. La Merrill MA, Vandenberg LN, Smith MT, Goodson W, Browne P, Patisaul HB, *et al.* Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. Nat Rev Endocrinol. 2020;16(1):45-57. doi:10.1038/s41574-019-0273-8.
27. Richter DU, Mylonas I, Toth B, Scholz C, Briese V, Friese K, *et al.* Effects of phytoestrogens genistein and daidzein on progesterone and estrogen (estradiol) production of human term trophoblast cells in vitro. Gynecol Endocrinol. 2009;25(1):32-38. doi: 10.1080/09513590802485020.
28. Guo L, Su J, Deng BW, Yu ZY, Kang LP, Zhao ZH, *et al.* Active pharmaceutical ingredients and mechanisms underlying phasic myometrial contractions stimulated with the saponin extract from *Paris polyphylla* Sm. var. yunnanensis used for abnormal uterine bleeding. Hum Reprod 2008;23:964–971.
29. Watcho P, Ngadjui E, Nkeng-Efouet PA, Nguelefack TB, Kamanyi A. Evaluation of in vitro uterotonic activities of fruit extracts of *Ficus asperifolia* in rats. Evid Based Complement Alternat Med 2011;783413
30. Amiera ZU, Nihayah M, Wahida IF, Rajab NF. Phytochemical characteristics and uterotonic effect of aqueous extract of Ficus deltoidea Leaves in the rat uterus. Pak J Biol Sci, 2014;17(9):1046- 1051.
31. Calixto JB, Yunes RA, Miguel OG, Rae GA. Effect of *Sebastiana schottiana* extracts on isolated smooth muscle contraction. Planta Med. 1986; 6:444.
32. Ekambaram G, Sampath Kumar SK, Joseph LD. Comparative study on the estimation of estrous cycle in mice by visual and vaginal lavage method. J Clin Diagn Res 2017;11(1):AC05–AC07.
33. Cora MC, Kooistra L, Travlos G. Vaginal cytology of the laboratory rat and mouse: Review and criteria for the staging of the estrous cycle using stained vaginal smears. Toxicol Pathol 2015;43(6):776–793. doi:10.1177/0192623315570300 39
34. Salami SA, Salahdeen HM, Obafemi AE, Murtala BA. Attenuation of impaired uterine contractile activity by ascorbic acid supplementation during early gestational variable stress exposure in Wistar rats. J Afr Assoc Physiol Sci 2020;8(1):16-22.
35. Umamageswari J, Balasubramanian S, Vijayarani K, Krishnakumar K, Preetha S. A simple and rapid staining technique to confirm mating in Wistar rats. J Entomol Zool Stud 2020;8(5):820–823. doi:10.22271/j.ento.2020.v8.i5l.7612
36. Chase MW, Christenhusz MJM, Fay MF, Byng JW, Judd WS, Soltis DE, *et al.* An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc 2016;181:1–20. doi:10.1111/boj.12385
37. Allen R, O'Brien BM. Uses of misoprostol in obstetrics and gynecology. Rev Obstet Gynecol. 2009;2(3):159-68.
38. Goldberg AB, Greenberg MB, Darney PD. Misoprostol and pregnancy. N Engl J Med. 2001;344:38–47.
39. Dabhadkar D, Zade V. Abortifacient efficacy of *Indigofera trifoliata* leaves extract on female albino rats. Asian J Pharm Clin Res. 2013;6(3):75-79.
40. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. CSH Protocols. 2008; 1:pdb.prot4986.
41. Schafer KA, Eighmy J, Fikes JD, Halpern WG, Hukkanen RR, Long GG, *et al.* Use of severity grades to characterize

histopathologic changes. Toxicol Pathol 2018;46(3):256–265
42. Savic I, Milovanovic P, Opric S, Ivanovic N, Oprić D. Expression of connexin-43 in surgical resections of primary tumor and lymph node metastases of squamous cell carcinoma and adenocarcinoma of the lung: A retrospective study. PeerJ. 2022;10:e13055. doi:10.7717/peerj.13055.
43. Sorensen IV, Fenger C, Winther H, Foged NT, Lademann U, Brünner N, *et al.* Characterization of anti-TIMP-1 monoclonal antibodies for immunohistochemical localization in formalin-fixed, paraffin-embedded tissue. J Histochem Cytochem 2006;54(10):1075-86.
44. Yedlinsky NT, Morgan FC, Whitecar PW. Anomalies associated with failed pregnancy and misoprostol termination. Obstet Gynecol. 2005;105:1203–1205.
45. Jing T, Shijian L, Jieqiong Y, Hongwanyu L, Weiya L, Cong Z. Decidualization and related pregnancy complications. Matern Fetal Med. 2022;4(1):24-35. doi:10.1097/FM9.0000000000000135.
46. Ng SW, Norwitz GA, Pavlicev M, Tilburgs T, Simón C, Norwitz ER. Endometrial Decidualization: The primary driver of pregnancy health. Int J Mol Sci. 2020;21(11):4092. doi: 10.3390/ijms21114092.
47. Chen J, Khalil RA. Matrix metalloproteinases in normal pregnancy and preeclampsia. Prog Mol Biol Transl Sci. 2017;148:87-165. doi: 10.1016/bs.pmbts.2017.04.001.
48. Cartwright JE, Fraser R, Leslie K, Wallace AE, James JL. Remodelling at the maternal-fetal interface: relevance to human pregnancy disorders. Reproduction. 2010;140(6):803-813. doi:10.1530/REP-10-0294.
49. Karamanos NK, Theocharis AD, Neill T, Iozzo RV. Matrix modeling and remodeling: a biological interplay regulating tissue homeostasis and diseases. Matrix Biol J Int Soc Matrix Biol. 2019;75–76:1–11.
50. Khan YH, Uttra AM, Qasim S, Mallhi TH, Alotaibi NH, Rasheed M, *et al.* Potential role of phytochemicals against matrix metalloproteinase-induced breast cancer: an explanatory review. Front Chem. 2021;8:592152. doi: 10.3389/fchem. 2020.592152.
51. Singer J, Angelini DJ, LaFontaine D, Cronin B, Howard ED. Recognition and treatment of post abortion complications. In: Angelini DJ, editor. Obstetric triage and emergency care protocols. New York (NY): Springer Publishing Company; 2017. p. 57-66.
52. Mitchell BF, Schmid B. Oxytocin and its receptor in the process of parturition. J Soc Gynecol Investig. 2001;8:122–133
53. Vrachnis N, Malamas FM, Sifakis S, Parashaki A, Iliodromiti Z, Botsis D, *et al.* Oxytocin and myometrial contractility in labor. In: Sifakis S, editor. From preconception to postpartum. Rijeka (Croatia): InTech; 2012. p. 243-254. Available from: http://www.intechopen.com/books/from-preconception-to-postpartum/oxytocin-and-myometrial-contractility-in-labor
54. Molnar M, Hertelendy F. Signal transduction in rat myometrial cells: comparison of the actions of endothelin-1, oxytocin, and prostaglandin F2 alpha. Eur J Endocrinol. 1995;133(4):467-74
55. Soloff MS, Jeng YJ, Copland JA, Strakova Z, Hoare S. Signal pathways mediating oxytocin stimulation of prostaglandin synthesis in select target cells. Exp Physiol. 2000;85:51S-58S. doi:10.1111/j.1469-445x.2000.tb00007.x
56. Zeeman GG, Khan-Dawood FS, Dawood MY. Oxytocin and its receptor in pregnancy and parturition: current concepts and clinical implications. Obstet Gynecol. 1997;89:873–883
57. Petraglia F, Imperatore A, Challis JR. Neuroendocrine mechanisms in pregnancy and parturition. Endocr Rev. 2010;31(6):783-816.
58. Huang Y, Ye D, Wu P, Huang Y, Zhang L, Zhou X, *et al.* Expression of cyclooxygenase-2 mRNA and identification of its splice variant in human myometrium obtained from women in labor. Journal of Huazhong University of Science, Technology, and Medical Sciences. 2005;25: 5–7.
59. Norman JE, Bollapragada S, Yuan M, Nelson SM. Inflammatory pathways in the mechanism of parturition. BMC Pregnancy Childbirth. 2007;7(Suppl 1):S7.
60. Tamura M, Deb S, Sebastian S, Okamura K, Bulun SE. Estrogen up-regulates cyclooxygenase-2 via estrogen receptor in human uterine microvascular endothelial cells. Fertil Steril. 2004;81(5):1351- 1356. doi: 10.1016/j.fertnstert.2003.09.076. PMID: 15136101.