



Effects of ethanolic extract of *lawsonia inermis* on pregnancy outcomes, fetal growth and the developing cerebral cortex of wistar rats

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Abstract

Background: Henna is predominantly consumed in the northern part of Nigeria by males to enhance fertility whereas the female counterparts ingest it to terminate pregnancies.

Objective: The aim of the study was to investigate the teratogenic potential of the ethanol leaf extract of *Lawsonia inermis* (Henna) on the fetal cerebral cortex and somatic indices like fetal weight with and without placenta, crown-rump length, placental weight, umbilical cord length and tail length of albino rats.

Materials and methods: Twenty adult female albino Wistar rats (150-200g) and eight adult male rats (190-220g) were purchased for this study. The female rats were divided into four groups labelled A-D. The experimental groups, B-D had maternal administration of the extract at 200, 400, and 600mg/kg body weight respectively. One-way ANOVA and Scheffes post-hoc test were used for data analysis and $p < 0.05$ was considered statistically significant.

Results: The mean number of fetuses across the treatment groups showed a significant difference from the low, medium to high dose groups respectively thus: 6.00 ± 0.45 , 3.60 ± 0.87 and 3.00 ± 0.32 when compared to the control group (6.40 ± 0.75) at $p < 0.05$. Thus, the number of fetuses decreased in a dose-dependent manner with the presence of resorption sites in the medium and high dose groups.

Conclusion: When the extract consumed is for abortifacient purposes and the dosage is not high enough for termination of the pregnancy, possible alterations may occur in the fetus, especially during the critical period of brain development. Therefore, henna has the potential to cause malformations in fetal cerebral cortex.

Keywords: Teratogen, *Lawsonia inermis*, henna, fetal, cerebral cortex, ethanol extract

1.0 Introduction

Lawsonia inermis is a branched, glabrous shrub or small tree usually found in Africa, Asia, and Australia^{1,2}. In the northern part of Nigeria, it is traditionally used as a tattoo dye for the festive seasons³. In Africa, the common names of *L. inermis* are Henna, Mehndi, Samphire, and Cypress shrub. However, in Nigeria, Hausas call it Lalle, Ibos call it Uhie inine while the Yorubas call it Laali⁴. The major phytochemical present in it is lawsone⁵. Henna leaves are reported to have many therapeutic effects which

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include analgesic; anti-viral; anti-parasitic; anti-trypanosomal; anti-dermatophytic; anti-fertility; tuberculostatic; and anti-cancer properties^{6,7,8}.

The cerebral cortex is sensitive almost throughout gestation⁹. The changes in the central nervous system function may be the most critical sign of pre-natal exposure to neurotoxic agents. The decrease in

mental capacity and delayed maturation following pre-natal exposure to teratogens are associated with alteration in the number and structures of neurons throughout the cerebral cortex¹⁰. The physiological organization of the human body as well as consciousness is determined by the central nervous system (CNS) which has neurons and neuroglial cells. The neuroglial cells include astrocytes, oligodendrocytes, ependymal cells, and microglial cells.

Astrocytes are one of the most common cells in the mammalian brain and they are distributed in every part of it¹¹. The neural cells undergo neurogenesis regulated by physiological, pathological, pharmacological, and environmental factors¹². Growth factors, cytokines, neurotrophins, and hormones play a key role in adult neurogenesis¹³. Neurogenesis occurs between the 6th to 11th day of gestation which is the critical period of brain development in Wistar rats.

Considering birth defects, the placenta as an 'incomplete' barrier can allow certain toxic substances to go through thus when fetuses are exposed, mostly from maternal sources; they exhibit adverse long-term neurological outcomes such as sensory, learning, and attention difficulties, behavioural issues, epilepsy, and cerebral palsy¹⁴. Empirically, over 80% of abortions occur during the first 12 weeks of pregnancy in humans and chromosomal abnormalities are responsible for at least half of such abortions. After the first quarter of gestation, both the abortion rate and the incidence of chromosomal abnormalities tend to be reduced. It has been suggested that the diagnosed rates of abortion are predicted to rise from 12% for women under 20 years to 26% for women over 40¹⁵. Although henna is being used as a medicinal herb for therapeutic purpose as stated in limited reports, there is lack of verifiable studies supporting its use as an abortifacient agent hence the need for this study.

2.0 Materials and Methods

2.1 Equipment

The pieces of equipment utilized for the study were wooden animal cages, Libror EB-330H electronic weighing balance, glass pipettes, sting and beakers, light microscope with glass slides, sliding Vernier caliper, plastic rule in centimeters, dissecting kit, hand gloves, dissecting table, phone camera, plastic

bottles, microtome and orogastric tube.

2.2 Sample collection and extract preparation

The *L. inermis* leaves used for the study were obtained from a market in Nguru village of Kano State. The leaves were identified and authenticated by a taxonomist at the Department of Botany in the University of Calabar, Nigeria. They were stored in the department's herbarium with the code, Bot/Herb/UCC/0176. *L. inermis* leaves were harvested from their stems manually, washed, and air-dried under room temperature for two weeks; afterward, they were ground into powdered form and used for the ethanol extract preparation. The extraction was done using hot extraction with 80% ethanol by using Soxhlet for 18 hours and the extract was filtered and vacuum dried (Delyvac). The semi-solid extracts were preserved at -20°C for further uses¹⁶. The process of extraction was carried out at the Chemistry department laboratory of the University of Calabar, Nigeria.

2.3 Animal Studies

Twenty adult female albino rats (150–200 g) and eight adult male rats (190–220 g) were purchased from the Animal House of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. They were kept in four well-ventilated wooden cages for two weeks of acclimatization. The cages were obtained from the Faculty of Basic Medical Sciences' Animal House, University of Calabar. The animals were fed with Growers' Feed obtained from Vital Feeds, Akim Market, Calabar. The rats were maintained under standard conditions and the animal room was well-ventilated with a temperature range of 25–27°C under day/night 12–12 hour photoperiodicity. The weights of these rats were taken before the commencement of the experiment and before harvesting the fetuses.

2.4 Experimental design

The twenty rats were randomly divided into four groups (A, B, C, and D) of five animals per group (n=5). They were caged and monitored for their heat (estrous) periods, ascertained by collecting vaginal smear with glass slides every morning (7–8 a.m) using Pasteur pipette with 10% normal saline. The female rats were mated during the estrous phase with sexually matured male albino rats in different

cages in a ratio of 2:1. To determine day zero of pregnancy, the presence of a tailed structure in the vaginal smear, representative of a vaginal plug when viewed under the light microscope at x100 and x400 magnification served as a marker for mating¹⁷.

Group A (Control): rat chow and distilled water orally for the same period as the experimental groups.

Group B (Low dose): rat chow, water, and 200mg/kg body weight of ethanol extract of *L. inermis*.

Group C (Medium dose): 400mg/kg body weight of the ethanol extract of *L. inermis*.

Group D (High dose): 600mg/kg body weight of the ethanol extract of *L. inermis*.

Groups B-D received the extract daily for six days from the 6th to 11th day of gestation through orogastric tube, once daily.

2.5 Morphological Studies

The fetuses were blot-dried and examined for gross morphological changes. The fetuses were weighed with and without placenta using Libror EB-330H electronic weighing balance, crown-rump length and tail lengths were measured using a ruler calibrated in millimeters and centimeters. The heads were decapitated, and brains (cerebral cortex) were removed, blotted dry and preserved in formal saline.

2.6 Histological study

The histological examination of the fetal cerebral cortex was done using haematoxylin and eosin staining technique for the cerebral cortical architecture according to Lorke's method¹⁸. The fetuses were fixed with 10% buffered formalin, later the skulls were dissected, and the brains were removed and preserved in 10% buffered formalin for 48 hours before tissue processing commenced.

2.7 Determination of Acute Toxicity Test

The dosage of *L. inermis* leaf extract was based on pre-determined LD50 of 2000mg/kg b.w obtained from previous studies^{16,19,20}.

2.8 Statistical Analysis

The data were analyzed using One-way ANOVA and Scheffes post-hoc test to determine significant differences between the control and the

experimental groups. All values were expressed as mean $\pm$ standard error of mean (SEM) and the level of significance for the study was expressed as $p < 0.05$.

3.0 Results

3.1 Somatic indices

3.1.1 Number of fetuses: The mean number of fetuses across the treatment groups showed a significant difference from the low dose, medium dose to high dose groups respectively thus: 6.00 ± 0.45 , 3.60 ± 0.87 and 3.00 ± 0.32 when compared to the control group (6.40 ± 0.75) at $p < 0.05$. The high dose group had relatively the lowest number of fetuses compared to the other groups. This showed that the birth rate was affected negatively with increase in dosage of *L. inermis* (Table 1)(Figure 1s).

3.1.2 Fetal weight with placenta: The mean fetal weights with placenta of the fetuses were 2.33 ± 0.09 g, 4.41 ± 0.35 g and 4.75 ± 0.46 g for the low, medium and high doses respectively. The medium and high dose were significantly higher when compared to the control group (1.86 ± 0.02 g) at $p < 0.05$ (Table 1).

3.1.3 Fetal weight without placenta: The mean fetal weights without placenta in low dose (1.76 ± 0.07 g), medium dose (3.71 ± 0.38 g) and high dose (4.14 ± 0.44 g) were significantly higher especially the medium and high dose groups when compared to the control group (1.42 ± 0.02 g) at $p < 0.05$ (Table 1).

Table 1: The effect of ethanol leaf extract of *L. inermis* on the fetal weight with and without placenta

Groups	No. of fetus	Fetal wt. + placenta	Fetal wt. - placenta
Control (A)	6.40 ± 0.75	1.86 ± 0.02	1.42 ± 0.02
Low dose (B)	6.00 ± 0.45	2.33 ± 0.09	1.76 ± 0.07
Medium dose (C)	3.60 $\pm 0.87^{*,a}$	4.41 $\pm 0.35^{*,a}$	3.71 $\pm 0.38^{*,a}$
High dose (D)	3.00 $\pm 0.32^{*,a}$	4.75 $\pm 0.46^{*,a}$	4.14 $\pm 0.44^{*,a}$

Values are expressed as mean + SEM, n = 5

* = significantly different from control at $p < 0.05$

a = significantly different from low dose at $p < 0.05$

b = significantly different from medium dose at $p < 0.05$

3.1.4 Placental weight: the mean placental weights of fetuses in the different experimental groups showed relatively no significant difference among groups when compared to the control at $p < 0.05$ (Table 2).

3.1.5 Crown-Rump length: the mean crown-rump length of low dose (4.30 ± 0.03 cm), medium dose (5.48 ± 0.17 cm) and high dose (6.05 ± 0.16 cm) groups were significantly higher than the control group (3.79 ± 0.03 cm). There was significant increase in the crown-rump length across the treatment groups with increase in dosage at $p < 0.05$ (Table 2).

3.1.6 Tail length: the mean tail length of medium dose (1.30 ± 0.07 cm) and high dose (1.68 ± 0.12 cm) groups were significantly higher than the control group (1.08 ± 0.01 cm) at $p < 0.05$ (Table 2).

3.1.7 Umbilical cord length: the mean umbilical cord length of the medium dose group (2.79 ± 0.07 cm) was significantly higher than that of low dose (2.17 ± 0.04 cm) and high dose (2.19 ± 0.07 cm) groups at $p < 0.05$. The mean umbilical cord lengths of the low and high dose groups were significantly lower than that of control group (2.63 ± 0.04 cm) at $p < 0.05$ (Table 2).

Table 2: The effect of *Lawsonia inermis* on somatic indices of the control and experimental groups

Group	Placental weight(g)	Tail length(cm)	Crown-rump length(cm)	Umbilical cord length(cm)
Control	0.46 ± 0.08	1.08 ± 0.01	3.79 ± 0.03	2.63 ± 0.04
Low dose	0.51 ± 0.02	1.18 ± 0.04	4.30 $\pm 0.10^*$	2.17 $\pm 0.04^*$
Medium dose	0.56 ± 0.05	1.30 $\pm 0.07^*$	5.48 $\pm 0.17^{*,a}$	2.79 $\pm 0.07^{*,a}$
High dose	0.44 ± 0.04	1.68 $\pm 0.12^{*,a,b}$	6.05 $\pm 0.16^{*,a,b}$	2.19 $\pm 0.07^{*,b}$

Values are expressed as mean $\pm$ SEM, n = 5.

* = significantly different from control at $p < 0.05$

a = significantly different from low dose at $p < 0.05$

b = significantly different from medium dose at $p < 0.05$

a = resorption site in medium dose group (400 mg/kg/b.w) and b = resorption site in high dose group (600 mg/kg/b.w)

3.3 Histological Analysis

The fetal cerebral cortices of the control and experimental groups (A-D) were fixed and processed using the haematoxylin and eosin (H & E) staining technique (Figure 1).

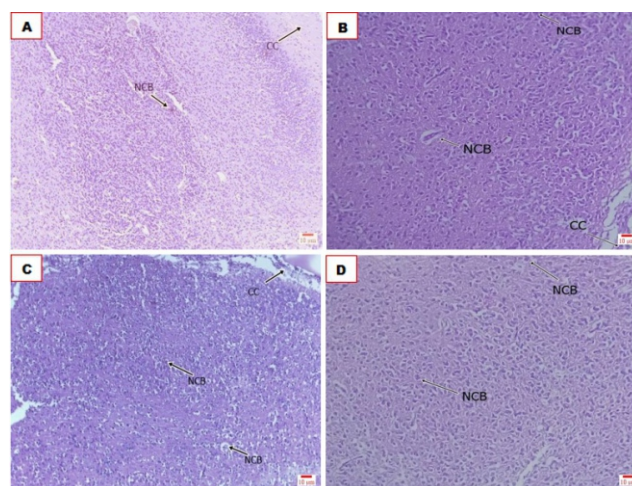


FIG 1: Representative photomicrographs of H&E-stained fetal cerebral cortices at x100 magnification. The control group (Figure 1a) showed densely populated neuronal cell bodies that have a uniform round blue nuclei with moderate amount of cytoplasm and fine chromatin patterns. The experimental groups (Figures 3b-d) showed uniform round blue nuclei but distinctly had the following i) Figure 1b showed slightly populated neuronal cell bodies with moderate amount of cytoplasm and fine chromatin patterns. ii) Figure 1c showed slightly populated neuronal cell bodies with more amounts of cytoplasm and fine chromatin patterns. iii) Figure 1d displayed fewer neuronal cell bodies with large amount of cytoplasm and fine chromatin patterns. NCB = neuronal cell body, CC = cerebral cortex, A = control group, B = low dose group, C = Medium dose group, D = high dose group

4.0 Discussion

The use of herbal medicines during pregnancy remains a significant public health concern, particularly in developing countries where traditional medicinal practices are widely accepted. Medicinal plants are frequently consumed for various reproductive purposes, including the maintenance of pregnancy, enhancement of fertility,

induction of labour and termination of unwanted pregnancies. However, the widespread perception that herbal preparations are safe because they originate from natural sources is not always supported by scientific evidence. Certain medicinal plants contain biologically active compounds capable of crossing maternal–fetal barriers, interfering with embryonic development and producing adverse reproductive outcomes.

Lawsonia inermis (Henna) is a medicinal plant traditionally used for cosmetic, therapeutic and reproductive purposes. In parts of Northern Nigeria, it has reportedly been consumed by women for pregnancy termination and by men for fertility enhancement. These ethnomedicinal applications raise important concerns regarding its reproductive safety, particularly when exposure occurs during the early stages of pregnancy. At this developmental period, embryogenesis, organogenesis and the establishment of major organ systems are highly sensitive to chemical and environmental disturbances.

The present study investigated the effects of ethanolic extract of *Lawsonia inermis* on pregnancy outcomes, fetal morphometric parameters and the histological architecture of the developing cerebral cortex in Wistar rats. The findings revealed evidence of embryonic resorption, alterations in fetal growth parameters and increased numbers of cells identified histologically as astrocytes within the fetal cerebral cortex. These observations suggest that maternal exposure to *L. inermis* during critical periods of gestation may adversely affect reproductive outcomes and fetal neurodevelopment.

One of the principal findings of the present study was the identification of embryonic resorption sites in the medium- and high-dose treatment groups. This observation suggests that exposure to ethanolic extract of *Lawsonia inermis* may compromise embryonic survival, particularly at higher administered doses. Embryonic resorption is an important indicator of prenatal developmental toxicity in experimental rodents and may result from disturbances in implantation, placental function, embryonic viability or maternal physiological homeostasis. Bello²¹ reported the traditional use of *Lawsonia inermis* for inducing first-trimester abortion and managing postpartum

haemorrhage. These reported applications suggest that the plant may possess pharmacologically active constituents capable of influencing uterine physiology. Uterotonic activity has been proposed as a possible mechanism underlying its reproductive effects. However, the involvement of beta-adrenergic signalling or other specific molecular pathways requires direct experimental confirmation.

The findings of the present study are broadly consistent with those described by Al-Snafi⁷, who reported abortifacient effects following the administration of hydroalcoholic extract of *L. inermis* to pregnant mice. In that report, intraperitoneal administration of 1 mg/kg and 10 mg/kg body weight from gestational days 1 to 17 was associated with pregnancy loss, evidenced by embryonic resorption sites.

Similarly, the present investigation demonstrated resorption sites in the medium- and high-dose groups, suggesting a possible association between extract exposure and embryonic loss. Nevertheless, differences in animal species, extract preparation, route of administration, treatment duration and administered doses should be considered when comparing the findings of both studies.

The occurrence of resorption sites in the treated animals may indicate that some embryos were unable to survive following exposure to the extract. However, the precise mechanism responsible for this outcome remains uncertain. Potential mechanisms include direct embryotoxicity, altered uterine contractility, impaired placental perfusion, oxidative stress and disruption of endocrine regulation during pregnancy. Importantly, the resorption represents the loss and subsequent degradation of embryonic or fetal tissue within the uterus. The observed findings therefore provide evidence suggestive of embryotoxic or abortifacient potential rather than definitive proof of a particular mechanism of pregnancy termination.

Macroscopic examination of the uteri revealed pigmented areas along the uteromesometrial border, which were interpreted as possible placental scars or sites of embryonic resorption. These areas appeared as yellowish to darkly pigmented regions and may reflect tissue remodelling and the accumulation of blood-derived pigments following embryonic demise. Drews et al.²² described the accumulation of

haemosiderin-containing cells within uterine tissues associated with placental regression and remodelling. Haemosiderin is an iron-storage pigment formed during the breakdown of haemoglobin and may accumulate within macrophages following local haemorrhage or tissue degeneration.

The pigmentation observed in the present study may therefore reflect haemorrhagic and inflammatory changes associated with embryonic resorption. However, macroscopic identification alone cannot conclusively establish the cellular composition or origin of these pigmented regions. Histological examination, supported by iron-specific staining such as Perls' Prussian blue, would be necessary to confirm haemosiderin deposition. Hashem et al.²³ described embryonic resorption as a complex pathological process involving disruption of the maternal-embryonic interface, degeneration of embryonic tissues and maternal inflammatory responses. Depending on the developmental stage and underlying cause, the process may involve apoptosis, immune-cell recruitment, tissue breakdown and clearance of embryonic remnants.

These mechanisms provide a possible explanation for the uterine changes observed in the present investigation. Nevertheless, without direct histological and molecular evaluation of the implantation sites, it is difficult to determine whether the observed resorption resulted primarily from embryonic toxicity, placental dysfunction or altered maternal uterine physiology. The identification of resorption sites in the treated groups is therefore an important finding that warrants further investigation using detailed placental histomorphometry, immunohistochemistry and molecular markers of apoptosis and inflammation.

Fetal morphometric assessment provides useful information regarding intrauterine growth and developmental progression. Crown-rump length and tail length are commonly employed as indicators of fetal growth in experimental developmental studies. In the present investigation, exposure to ethanolic extract of *Lawsonia inermis* was associated with increased fetal crown-rump length and tail length across the treatment groups. This finding suggests that the extract may influence fetal growth patterns during gestation.

The observation contrasts with the findings of Abebe et al.²⁴, who investigated the developmental toxicity of hydroethanolic leaf extract of *Syzygium guineense* in pregnant albino Wistar rats. In their study, pregnant animals received 250, 500 and 1,000 mg/kg body weight of the extract during gestational days 6–12. Embryonic assessments conducted on gestational day 12 revealed significant reductions in crown-rump length, somite number and morphological developmental scores at the highest administered dose.

In contrast, the present study, in which pregnant rats were treated between gestational days 6 and 11 and fetuses examined on gestational day 20, demonstrated increased crown-rump length and tail length following exposure to *L. inermis*. The differences between these findings may be attributable to variations in plant phytochemical composition, administered doses, gestational exposure periods, experimental protocols and developmental stages at which measurements were obtained.

Furthermore, the biological significance of increased fetal length requires careful interpretation. Although increased crown-rump length may indicate altered growth, it does not necessarily reflect improved fetal health or normal developmental progression. Disproportionate fetal growth may occur alongside structural abnormalities, disturbances in organ maturation or other developmental alterations. The coexistence of increased fetal morphometric measurements and embryonic resorption in the present study suggests that exposure to *L. inermis* may produce different effects on embryonic survival and the growth of surviving fetuses.

It is also possible that the surviving fetuses represent a subset with greater tolerance to the administered extract. Consequently, the increased fetal lengths should be interpreted in conjunction with fetal body weight, litter size, implantation number, resorption frequency and the presence or absence of congenital abnormalities. Additional studies are required to establish whether the observed changes represent a direct growth-modulating effect of the extract or an indirect consequence of altered intrauterine conditions.

The developing cerebral cortex is particularly vulnerable to disturbances arising from exposure to

biologically active substances during gestation. Normal cortical development involves tightly regulated processes of neural progenitor proliferation, neuronal differentiation, migration, synaptogenesis and glial development. Disruption of these processes may have lasting consequences for cerebral architecture and neurological function. Histological examination of the fetal cerebral cortex in the present study revealed an apparent dose-dependent increase in cells identified as astrocytes in the treatment groups. This observation suggests that maternal exposure to ethanolic extract of *Lawsonia inermis* may be associated with alterations in the cellular composition of the developing cerebral cortex.

Astrocytes are important glial cells that contribute to neuronal support, extracellular ion regulation, neurotransmitter homeostasis, metabolic regulation and the maintenance of neural tissue integrity. They also participate in the response to central nervous system injury through changes in their morphology, molecular expression and cellular activity. An increase in astrocytic profiles may therefore represent a response to altered tissue conditions or cellular stress. However, the significance of this finding must be considered within the developmental context of the fetal brain.

During prenatal development, astroglial populations undergo progressive differentiation and maturation. Consequently, an apparent increase in astrocytic cells may reflect changes in glial differentiation, developmental timing or tissue response rather than necessarily indicating reactive astrogliosis. Although the dose-related pattern observed in the present study raises concern about possible neurodevelopmental toxicity, conventional histological examination alone cannot conclusively establish astrocyte proliferation, neuronal degeneration or impaired neurogenesis.

The use of immunohistochemical markers such as glial fibrillary acidic protein (GFAP) would assist in identifying astroglial cells and evaluating changes in their distribution and morphology. Similarly, Ki-67 could be used to investigate cellular proliferation, while markers such as doublecortin and NeuN may provide additional information about immature and mature neuronal populations, respectively. Taken together, the findings suggest that gestational exposure to ethanolic extract of

Lawsonia inermis may influence the developing cerebral cortex, represent genuine neurotoxicity and whether they have functional consequences for postnatal neurological development.

Previous experimental investigations have documented reproductive toxicity associated with certain preparations of *L. inermis*. A review by Batiha et al.²⁵ identified reports of pregnancy loss and developmental toxicity following exposure to henna extracts. These findings support the need for careful evaluation of the plant's safety during pregnancy. Of particular concern, is the possibility that exposure to doses insufficient to cause pregnancy loss may nevertheless influence the development of surviving fetuses. The coexistence of embryonic resorption and altered fetal cerebral histology in the present investigation raises this possibility, although a direct causal relationship between the two findings has not been established.

5.0 Conclusion

The present study demonstrated that maternal exposure to ethanolic extract of *Lawsonia inermis* during early gestation was associated with embryonic resorption, increased fetal crown-rump and tail lengths, and alterations in the cellular appearance of the developing cerebral cortex of Wistar rat fetuses. The occurrence of embryonic resorption sites, particularly in the medium- and high-dose groups, suggests possible embryotoxic effects of the extract. Furthermore, the apparent dose-dependent increase in astrocytic profiles within the fetal cerebral cortex raises concerns about potential disturbances in normal neural development. Although these findings suggest that *L. inermis* may possess reproductive and developmental toxicological properties, the precise cellular and molecular mechanisms remain unclear. The study therefore provides preliminary evidence that gestational exposure to ethanolic extract of *Lawsonia inermis* may adversely influence embryonic survival and fetal cerebral development.

Ethical approval

All authors hereby declare that the experiment has been examined and approved by the Faculty Research Ethical Committee of the Faculty of Basic Medical Sciences (FAREC-FBMS), University of Calabar and have therefore been performed in

accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The Ethical Approval Number - 125ANA3821 was issued to conduct the research and all the guidelines of the ethical committee were followed to the latter during the research.

Competing interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the outcome of the study.

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