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PRENATAL EXPOSURE AND TOXICITY OF AQUEOUS LEAF EXTRACT OF *ASPILIA AFRICANA* ON PLACENTA OF ALBINO WISTAR RAT FOETUSES

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ABSTRACT

Background: It has been estimated that over 80% of African population uses plant (herbs) regimen for treatment and control of disease due to it safety, availability, and effectiveness. In the present study, Aspilia africana one of the major herbs used by Nigerians to treat many ailments such as; wound healing, stoppage of bleeding, cough, gonorrhoea, feverish headache, stomach troubles was investigated. **Objective**: This study examined the prenatal exposure and toxicity of aqueous leaf extract of Aspilia africana on the placenta of foetal wistar rats. **Materials and methods**: Twenty adult female rats weighing between 190 - 205g was divided into four groups labeled control, low dose, medium dose and high dose, with each consisting of five rats. Pregnancy was induced by caging the female rats with sexually matured males. The presence of vaginal plug and tail structures in the vaginal smear the following morning confirmed coitus and it was regarded as day 0 of pregnancy. Control group was given distilled water. Low dose group received 750mg/kg body weight of aqueous leaf extract of Aspilia africana, medium dose and high dose groups received 1000mg/kg, and 1250mg/kg body weight of aqueous leaf extract of Aspilia Africana respectively. The administration was done orally with the aid of orogastric tube on days 7-11 of gestation. On the day 20 of gestation, the rats were sacrificed and the placenta extracted, fixed in formal saline and process for histological studies. **Result**: Histological observations of the placenta showed hyperemia in the labyrinth interhemal membrane, proliferation of maternal sinusoids, hypertrophy of fetal capillary, apoptosis of spongiotrophoblasts, degeneration and necrosis of the trophoblast. These observations were more severe in sections from rats whose mother received 1250mg/kg of Aspilia africana. **Conclusion**: The result suggests that aqueous leaf extract of Aspilia africana may be teratogenic to the developing placenta of Wistar rats and is dose dependent.

KEYWORDS: Apoptosis, Aspilia Africana, Interhemal membrane, Placenta, Foetus.

INTRODUCTION

Placenta is an organ that allows the exchange of nutrients and metabolic products between the mother and its fetus to ensure proper fetal growth (Desforges and Sibley, 2010). Placenta contains highly specialized trophoblast cells that form a barrier between the maternal uterus and the fetus (Knipp et al., 1999). Maternal blood supply (Hemberger and Cross, 2001) provides an adequate balance of nutrients, growth factors and hormones (Prater et al., 2008) for the growth and maintenance of the placenta. With few exceptions, most of drugs that are ingested by a pregnant woman during pregnancy can cross the placenta and reach the fetus. Whether knowingly or not, a mother is exposed to several drugs during pregnancy; the number can range from one to as many as six to eight drugs (Ostrea et al., 1987; Brocklebank et al., 1978; Doering and Stewart, 1978).

The human placenta is hemomonochorial, meaning that only one chorionic cell layer exists between maternal and fetal bloods (Casanueva and Fernando, 2003), thereby allowing nutrient uptake, waste elimination and gas exchange via the mother's blood supply (Wang, 2010). Adverse pregnancy situations, such as those involving maternal diabetes or obesity, can increase or decrease levels of nutrient transporters in the placenta resulting in overgrowth or restricted growth of the fetus (Kappen and Kruger, 2012). Research has shown that paternal exposure to some herbal plants could result in adverse outcomes in health of the neonates (Macías-Peacok et al., 2009; Nordeng and Havnen, 2004). Herbs are supposed to be safe but many unsafe and fatal side effects have been reported (Izzo, 2004). These could be direct toxic effects, allergic reactions, effects from contaminants and/or interactions with drugs and other herbs (Seth and Sharma, 2004).

Aspilia africana is widely used in ethno medical practice in Africa for its ability to stop bleeding, even from a severed artery and also aids in rapid healing of wounds and sores as well as in the management of problems related to cardiovascular diseases (Dimo et al., 2002).

Aspilia Africana has been classified among substances with a low potential for toxicity, with an LD_{50} averaging 6.6g/Kg body weight (Hanna and Niemetz, 1987). *Aspilia africana* is used in herbal medicine to treat various infections of bacterial origin such as gonorrhea, stomach trouble and corneal opacity (Adeniyi and Odufowora, 2000; Akinlabi, 2016).

Despite the countless number of functions of the placenta during the development of the foetus, it is also recognized as a target for toxic actions of some materials (Foster et al., 2008). Foreign compounds may interfere with placental function at many levels and any deviation from normal development may constitute a potential threat to placental function normal development may constitute a potential threat to placental function, resulting in preterm delivery, congenital malformation, or abortion (Myllynen et al., 2005). Since there is little or no research on the prenatal exposure and toxicity of aqueous extract of *Aspilia africana* on placenta in Albino wistar rat foetus, hence this study.

MATERIALS AND METHODS

Breeding of Animals

Twenty (20) adult female wistar rats used for the research work were obtained from Faculty of Basic Medical Sciences Animal House, University of Calabar, Calabar and bred in the animal house of the Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria in well ventilated wooden cages with iron nettings under hygienic conditions at 25 ± 2^0C and a relative humidity of 45–50% throughout the duration of the experiment. The animals were fed daily and regularly with grower mesh and water were given *ad libitum*.

Extract Preparation

Fresh leaves of *Aspilia Africana* were picked at the University of Calabar farm, Calabar, Cross River State of Nigeria. The plant was identified and authenticated at the Botany Department, University of Calabar, Calabar. The harvested fresh leaves were washed with clean water to remove dirt and air dried for two weeks. The dried leaves were homogenized with the aid of electric blender into fine powder in New Chemistry Laboratory, Department of Chemistry University of Calabar. One hundred and seventy-six grams (176g) of powdered leaves was soaked in one thousand two hundred millilitres (1200mls) of distilled water for 48hours in the research laboratory of the Biochemistry Department of the University of Calabar, Calabar. The filtered was obtained from the solution using Whatman's No 1 filter paper and evaporated dryness in an air-dry oven at 40^0C, the residue of the extract obtained in form of thick-semi solid paste was stored in a capped bottle and kept in a desiccators (Obembe et al., 2010).

Experimental Protocol

Twenty (20) albino wistar rats weighing about 190—205 grams were randomly selected and divided into four groups labelled control, low dose, medium dose and high dose, with each group consisting of 5 rats. The oestrous cycle of the animals was determined by daily vaginal lavages and at oestrous each rat was caged overnight with a sexually male rat of the same strain. The presence of vaginal plug and tail-like structures in the vaginal smear, the following day confirms coitus signifying day zero of pregnancy.

Extract Administration

Control: The control rats were given grower mesh and distilled water only.
Low Dose: The rats were administered with 750mg of the aqueous extract of *Aspilia Africana* per kilogram body weight.
Medium Dose: The rats were administered with 1000mg of the aqueous extract of *Aspilia Africana* per kilogram body weight.
High Dose: The rats were administered with 1250mg of the aqueous extract of *Aspilia Africana* per kilogram body weight.

The administration was done orally through orogastric intubation from days 7 - 11 of gestation respectively. The rats were euthanize on the 20 day of gestation using chloroform inhalation method. The fetal brains were excised, blotted with filter paper and immediately fixed in 10% formal saline for 48hours. Thereafter, the placenta was excised to process for histological studies.

Ethical consideration

All experimental investigations were done in compliance with humane animal care standard outlined by the Faculty of Basic Medical Sciences Committee on animal use and care, Universiity of Calabar and approval was given to carry out this research.

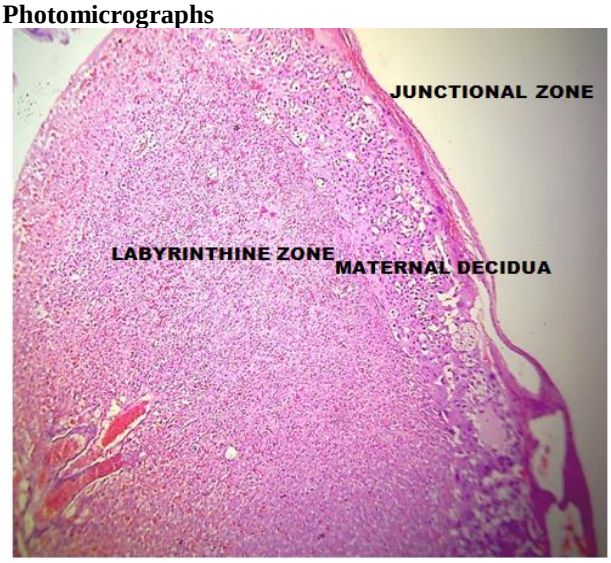
RESULTS

Histological Observations

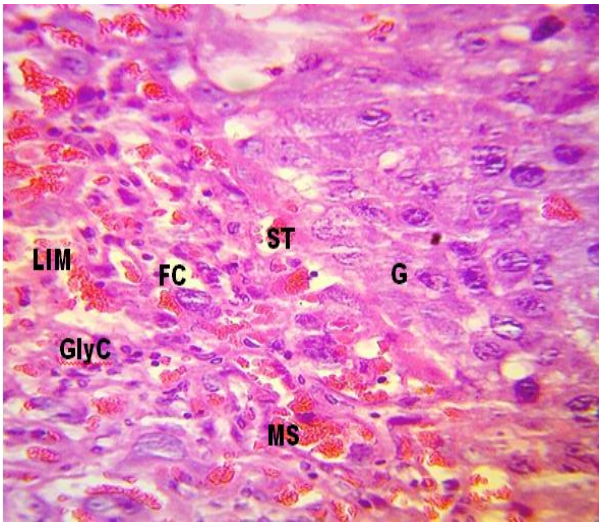
From the histological observation of sections of control group after staining with Haematoxylin and Eosin, different zones of the placenta were observed. The junctional zone, labyrinthine zone and maternal decidua. In the labyrinthine zone the maternal sinusoid, fetal capillary, spongiotrophoblast, labyrinth interhemal membrane and glycogen cell were observed. In the Junctional zone the trophoblastic giant cell were observed (Plate 1). Section of the placenta of low dose group that their mothers received 750mg/kg of *Aspilia africana* extract showed a reduction in thickness of trophoblastic giant cells (G), Slight proliferation of maternal sinusoids to the junctional zone (MS and JZ), hypertrophy of the fetal capillary(FC), Slight hyperemia of the labyrinth interhemal membrane (LIM). There were no noticeable changes in the glycogen cells (GlyC) and Spongiotrophoblast (ST), (Plate 2). Sections of the placenta of medium dose group that were administered 1000mg/kg showed decrease in number and reduction in thickness of trophoblastic giant cells (G), proliferation of maternal sinusoids to the junctional zone (MS and JZ)

and apoptosis of spongiotrophoblasts (ST), hyperemia of labyrinth interhemal membrane (LIM). No noticeable changes in the glycogen cells (GlyC). (Plate 3). Sections of the placenta in high dose group whose mothers received 1250mg/kg of *Aspilia africana* extract showed degeneration and necrosis of the trophoblastic giant cell (G), apoptosis of spongiotrophoblasts (ST), massive proliferation of maternal sinusoids to the junctonal zone (MS and JZ), Hypertrophy of the fetal capillary (FC), hyperemia of the labyrinth interhemal membrane(LIM). No noticeable changes in the glycogen cells (Plate 4).

Photomicrographs

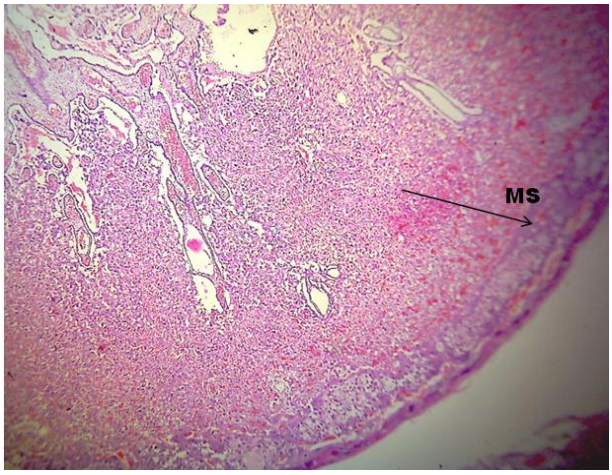


A.



B.

Plate 1: Photomicrographs (A & B) showing placenta of the control group. A part shows the Junctional Zone (JZ), Maternal Decidua (MD), Labyrinthine zone (LZ). The B part shows the Fetal capillary (FC), Maternal sinusoid (MS), Labyrinth interhemal membrane (LIM), Trophoblastic giant cell (G), Glycogen cell (GlyC), Spongiotophoblast (ST). Mag X100 and X400 H&E.



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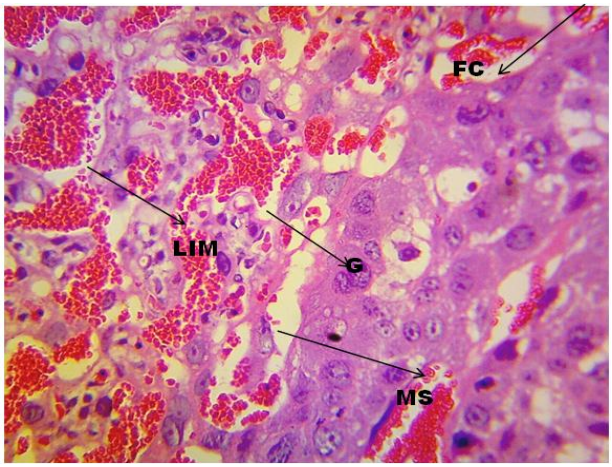
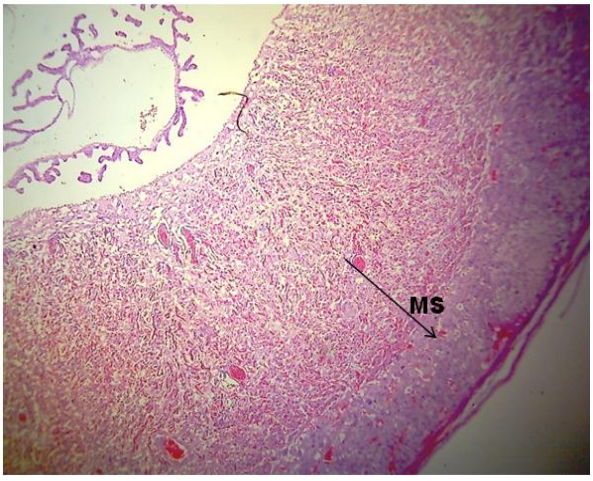


B.

PLATE 2: Photomicrograph (A & B) showing placenta in low dose treated with 750mg/kg of *Aspilia africana* leave extract showing the following structures (FC, MS, G, GlyC, ST). Reduction in thickness of Trophoblastic giant cells (G), Slight proliferation of maternal sinusoids to the junctional zone (MS and JZ), hypertrophy of the fetal capillary (FC), Hyperemia in the labyrinth interhemal membrane (LIM). Mag X100 & X 400 H&E.



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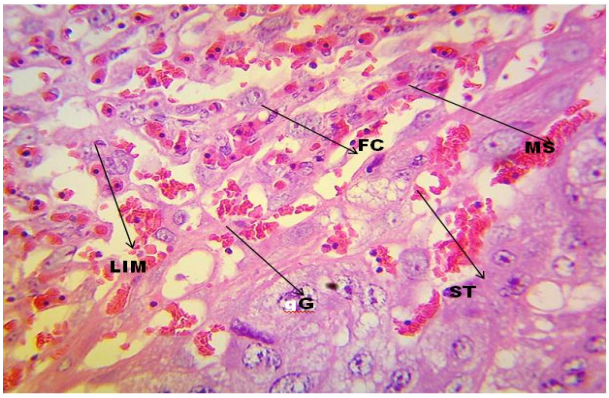
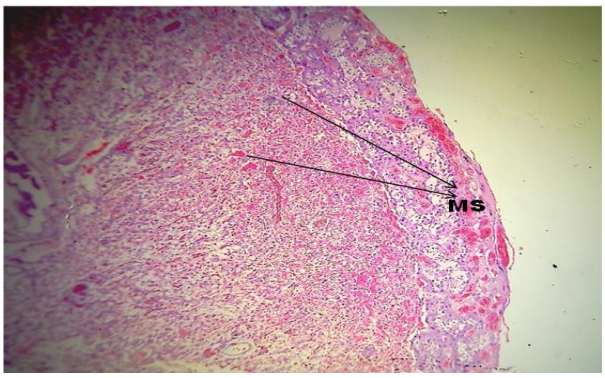
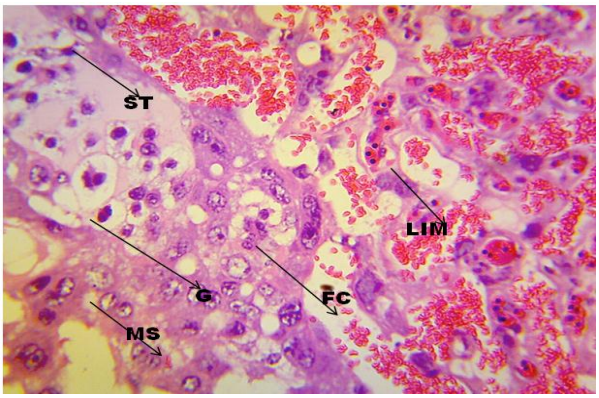


B.

PLATE 3: Photomicrograph (A & B) showing placenta in medium dose treated with 1000mg/kg of *Aspilia africana* leave extract showing the following structures (FC, MS, G, GlyC, ST). Decrease in number and reduction in thickness of Trophoblastic giant cells (G), Proliferation of maternal sinusoids to the junctional zone (MS and JZ), Hyperemia in the labyrinth interhemal membrane (LIM). Apoptosis of Spongiotrophoblasts (ST). Mag X100 & X400 H&E.



A



B

PLATE 4: Photomicrographs (A &B) of the placenta in high dose treated with 1250mg/kg of leave extract of *Aspilia africana* showing degeneration and necrosis of the trophoblastic giant cell (G), Apoptosis of Spongiotrophoblasts (ST), massive proliferation of maternal sinusoids to the junctonal zone (MS and JZ), Hypertropy of the fetal capillary (FC), Massive hyperemia in the labyrinth interhemal membrane (LIM). Mag X 100 and 400 H&E.

DISCUSSION

The placenta is a temporary structure unique to pregnancy functions to sustain and protect the fetus until birth (Page et al., 2002). It obtains its metabolic, immunological requirements (Vora et al., 2009; Chang, 2009) and secretory functions to support fetal development. The placenta is attached to the uterus, and the fetus is connected to the placenta via the umbilical cord (Karadeniz et al., 2007). The human placenta is hemomonochorial, meaning that only one chorionic cell layer exists between maternal and fetal bloods (Casanueva and Fernando, 2003), thereby allowing nutrient uptake, waste elimination and gas exchange via the mother's blood supply (Wang, 2010).

Findings from this study show that administration of different concentrations of the *Aspilia africana* extract in the rats produce significant changes in the placental histology. It has been in the demonstrated that herbal toxicity clearly represents serious human health threat and is an important issue that should be tackled (Chen et al., 2011).

In this present study, the placental trophoblastic giant cells in the treated rats with different concentration of *A. africana* were poorly developed. Similar results have been reported by researchers using different plant extract, *Carthamus tinctorius* L. from the asteraceae family (Louei and Salati, 2012). The results showed that treatment with 1.4 and 2.8 mg/kg *C. tinctorius* extract caused reduction in the trophoblastic giant cells ratio and increasing in the proportion of labyrinthine interhemal membrane (LIM). Moreover, the size of the labyrinthine zone per whole placenta, weight, diameter, and thickness of the placenta in the mice administered with 1.4 and 2.8 mg/kg *C. tinctorius* extract became lower than those of controls ($p<0.05$). It was thereby concluded in the study that treatment with *C. tinctorius* extract in doses of 1.4 and 2.8 mg/kg induces toxic changes in the placental structure so caution should be paid to popular consumption of this plant both as an alternative medicine and as a food additive. (Louei and Salati, 2012).

It can be noted during the experiment as the doses of the extract of *A. africana* been administered to the placenta tissue increases the trophoblastic giant cells and spongiotrophoblast showed degeneration and necrosis. This is in line with a study which was carried on the prenatal exposure of female albino rats to Aloe barbadensis on the placenta by Rengin et al, (2008), the result showed degenerative changes on the trophoblastic giant cells and spongiotrophoblasts of the placenta after administration of high dose of the extract.

Necrosis of the trophoblastic giant cells and spongiotrophoblast was very pronounced in the placenta tissue administered with high dose of 1250mg/kg of *Aspilia africana* extract. When stained it showed thinning and white spots which is in line with Satoshi et al. (2011) research on toxicological pathology in the rat

placenta. Studies have shown that placental Necrosis is induced by things as Valproate (Khera, 1992), chlorpromazine (Singh and Padmanabhan, 1980), glucocorticoid (Graf et al., 1989), streptozotocin (Padmanabhan et al., 1988), cadmium (Di Sant' Agnese et al., 1983), ethanol (Akay and Kockaya, 2005), lead acetate (Fuentes et al., 1996), diethylstilbestrol, estrogen, tobacco, adrenomedullin antagonist, cocaine and vitamin E-deficiency (Satoshi et al., 2011).

It has been suggested that trophoblastic giant cells participate in a number of processes essential to a successful pregnancy including blastocyst implantation, remodeling of the maternal decidua and secretion of hormones that regulate the development of both the fetal and maternal of the placenta (Ogren and Talamantes). It is also known that trophoblasts in the fetal part of the placenta are a common toxicological target tissue for some drugs and chemicals, because they have high proliferative activity and constitute a major structural component of the fetal part of the placenta (Satoshi et al., 2011).

In the study of histological and morphological characteristics of placenta in the rats administrated with Glycyrrhiza glabra extract was investigated. The obtained results showed that in the G. glabra-treated rats compared to control, trophoblastic giant cells were significantly decreased in the number and size. In addition, massive hyperemia was seen in the labyrinth interhemal membrane compartment of placenta in the G. glabra treated rats. Also, significant increase in the placental weight as well as in the placental index was found in the treated groups in comparison to the control rats. From this study, it was concluded that G. glabra extract administration has harmful effects on the placental structure and therefore popular consumption of this plant should be reconsidered (Ali, 2013).

In the current work hyperemia was seen in the labyrinth interhemal membrane compartment of the rats administered with the aqueous extract of *Aspilia africana*. The hyperemia of the LIM was not dose dependent as it was seen in the rats administered with 750mg/kg dose, 1000mg/kg dose and 1250mg/kg dose. The intergrity of the LIM is a critical parameter for the placental (Coan et al., 2004). Thus, in the present study, massive hyperemia of LIM in the placentas of *A. africana* treated rats, confirms an abnormality in the maternal-fetal blood barrier. These changes are associated with reduced nutrient supply from the maternal to the fetal circulation (Laurie et al., 1992, Masashi et al., 1992) and are in line with significant decrease in the fetal weight (Ali, 2013).

Flavonoid which is one of the chemicals found in *Aspilia africana* is also known for its anti-cancer properties (Pietta, 2000), might be responsible for the apoptosis of the placenta, another studies revealed that anti-cancer drugs induces trophoblastic apoptosis (Satoshi et al., 2011). Although mechanism (s) of *A. africana* toxic effects on the placentas is not clear but the leaf of this plant contains different active components including alkaloids, saponins, tannins flavonoids and phenol (Ekaiko et al., 2016) that could be responsible for these changes.

Conclusively, this research work shows that the consumption of *A. africana* leave extract may have adverse effect on the histology of the placenta of the developing foetus of rat. This histological alteration may affect the functions of placenta. Therefore it is advisable that the consumption of *Aspilia africana* in general should be reduced especially in pregnant women.

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