

**TERATOGENIC EFFECT OF AQUEOUS LEAF EXTRACT OF *ASPILIA AFRICANA* ON  
FRONTAL CORTEX OF ALBINO WISTAR RATS**

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**ABSTRACT**

*Aspilia Africana* is one of the major herbs being used by many Nigerians to treat many ailments such as; wound healing, stoppage of bleeding, cough, gonorrhoea, feverish headache, stomach troubles and also help in easy delivery and lactation. This study was to examine the teratogenicity of aqueous leaf extract of *Aspilia Africana* on frontal cortex of foetal wistar rats. Twenty adult female rats weighing between 190 - 205g were used for this study. The rats were divided into four groups labelled 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 was regarded as day zero (0) of pregnancy. Control group was given distilled water. Low dose, medium dose and high dose groups received 750mg, 1000mg and 1250mg of aqueous leaf extract of *Aspilia Africana* per kilogram body weight orally with the aid of orogastric tube respectively on days 7-11 of gestation. On the day 20 of gestation, the rats were sacrificed and the foetal brain was fixed in formal saline and later processed for histological studies. Histological observations of the frontal cortex showed hyperplasia and hypertrophy especially in the subventricular and ventricular layers of the low dose. Distortion of cortical, intermediate and subventricular layers of the medium dose group; and complete distortion of the layers of the frontal cortex in the high dose group characterised by numerous vacuolations and cellular degenerations. Blood vessels appeared tiny and almost absent in all the treated groups. The result suggests that aqueous leaf extract of *Aspilia Africana* may be teratogenic to the developing frontal cortex of wistar rats and is dose dependent.

**KEYWORDS:** Teratogenicity, *Aspilia africana*, aqueous extract, frontal cortex, foetuses.

**INTRODUCTION**

Plant materials have been used as sources of medical compounds in ethno medical practice and also in most rural communities in developing countries had played a dominant role in maintenance of human health (Mathews et al., 1999). World Health Organization defines herbal medicine as herbs, herbal materials, herbal preparations and finished herbal products, that contain as active ingredient parts of plants, or other plant materials or combinations (WHO).

In Benin City in Nigeria, researchers reported that pregnant women used both traditional herbal medicine and pharmaceutical drugs with the highest prevalence of concomitant use among nulliparous mothers (Gharoro and Igbafe, 2000). Although herbal medicine are natural, not all are safe to use during pregnancy as the herbal extract may get to the fetus by crossing the placenta affecting the fetus directly or indirectly by causing damage, abnormal development leading to birth defects or death, indirectly by altering the placenta function by causing constriction of blood vessels thus reducing

oxygen and nutrient supply. Few studies have been done to measure the effects of various herbs on pregnant women or foetuses (Bentil, 2015).

One of the primary functions of the brain is to respond adaptively to changes in the environment, thus environment may have a major impact on brain function. The prefrontal cortex, more than any other region of the brain, governs complex adaptive responses to changing environmental demands. The prefrontal cortex plays a role in a variety of cognitive and executive processes, including working memory, decision-making, inhibitory response control, attentional set-shifting and the temporal integration of voluntary behaviour (Jeffery et al., 2004). In adults, as well as children, there is a high degree of variability in cognitive functions that rely on PFC, including working memory (Vogel & Mechizawa, 2004; Vogel et al., 2005). Prefrontal cortex (PFC) takes over two decades of experience and growth to reach its full maturity (Casey et al., 2000; Fuster, 2002). Several studies have showed that the prolonged period of development makes the PCF particularly sensitive to

environmental influences (Andersen & Teicher, 2008; Crews *et al.*, 2007).

*Aspilia africana* is one of the many indigenous plants used by tradomedical practitioners in Nigeria to cure certain illness. It is known as organgila in Ibo, Tazalian in Hausa, Yungung in Yoruba and Edemedong in Efik (Single, 1965). It is a semi woody herb occurring throughout the regions of the Savannah and tropical Africa on Wastelands (Hutchinson, 1962; Burkill, 1985). The phytochemical analysis of the plant reveals that it has high crude oil protein content (Burkill, 1985). The leaf of *Aspilia africana* is very rich in alkaloids and saponins which are known to have antimicrobial activities (Duke, 1992). It contains flavonoids which are super antioxidants that provide protection against oxidative cell damage (Salah *et al.*, 1995) and against allergies, viruses ulcers and inflammation (Harborne, 1984). *Aspilia Africana* has been reported to possess antimicrobial, haemostatic (Achonye, 1976), anti-inflammatory (Okoli *et al.*, 2007) and anti-fertility (Eweka, 2008) activity but there is little or no work carried out to investigate the effect of *Aspilia Africana* on the fetal frontal cortex. Hence, this study carried out to investigate the teratogenic effect of aqueous leaf extract of *Aspilia Africana* on the frontal cortex of the albino Wistar rats fetuses.

## 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  $25\pm 2^{\circ}\text{C}$  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*.

### Ethical consideration

Approval was given by the Faculty of Basic Medical Sciences Committee on animal use and care, University of Calabar to carry out this research work following laid down rules and guidelines of the institution in the use of medicinal plants and animal models.

### 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  $40^{\circ}\text{C}$ , 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 frontal cortex was excised to process for histological studies.

## RESULTS

### Histological Observations

In the control group, five layers were observed following Haematoxylin and Eosin staining. These include: Marginal zone (MZ), cortical zone (CZ), intermediate zone (IZ), subventricular zone (SZ) and ventricular zone (VZ), these layers were all well-define, blood vessels (BV) were present in almost all the layers especially that of the ventricular zone (VZ) (Plate 1 A/B).

The frontal cortex of low dose group that were administered 750mg/kg of *Aspilia a.* extract showed all the layers of the developing cortex. There was hyperplasia and also hypertrophy in the subventricular

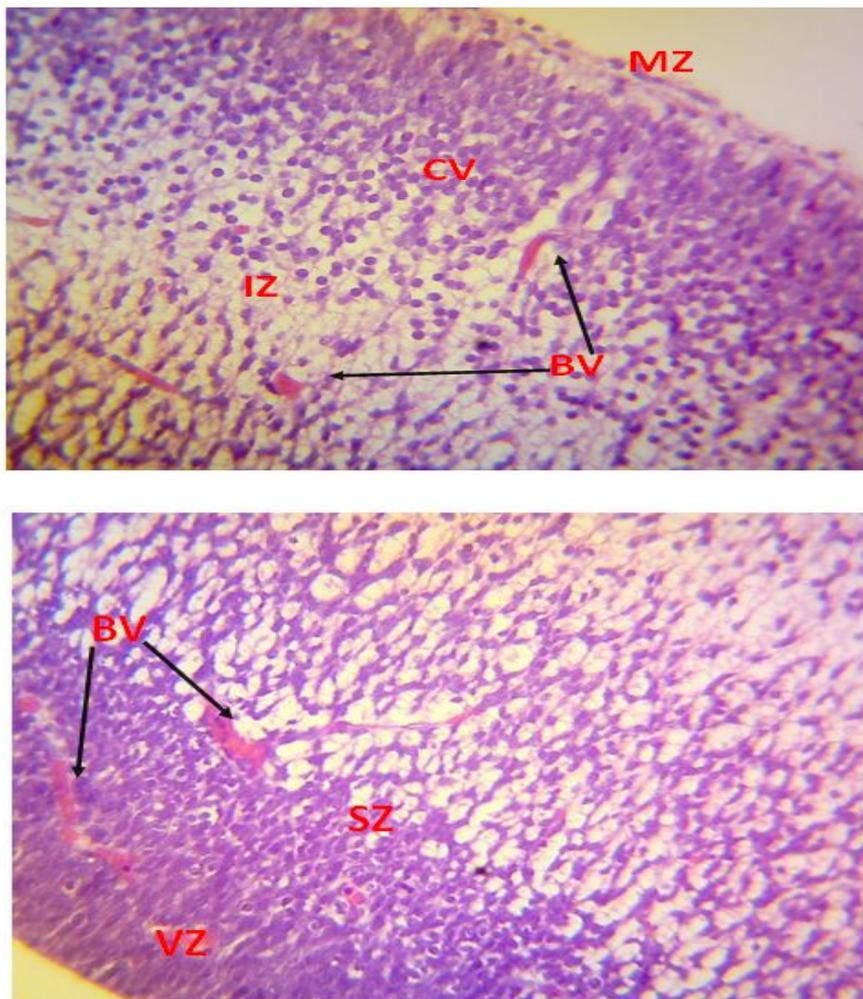
zone (HHSZ) and ventricular layer compare to control group. There was reduction in the intermediate and cortical zones as compare to the control group while cells in the marginal zone of low dose were slightly distorted (Plate 2).

The medium dose group that were administered with 1000mg/kg *Aspilia a.* extract showed some level of distortion. The cortical, intermediate subventricular and ventricular layers were highly distorted and there was

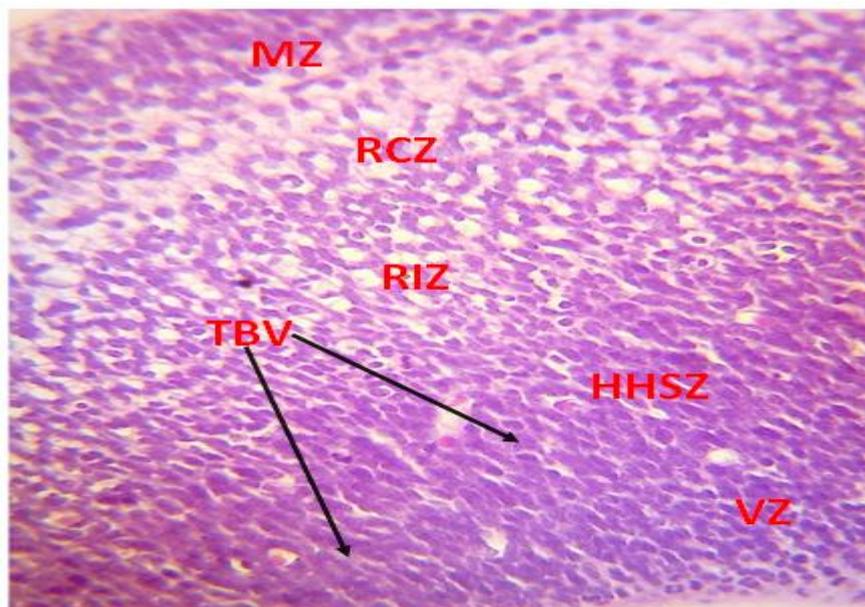
hypoplasia of cells in the ventricular zone when compare to the normal group (Plate 3).

There was complete distortion of the layers in the frontal cortex of animals in the high dose group administered with 1250mg/kg of *Aspilia Africana* leave extract. There was reduction in the ventricular and subventricular layers with fewer blood vessels (Plate 4).

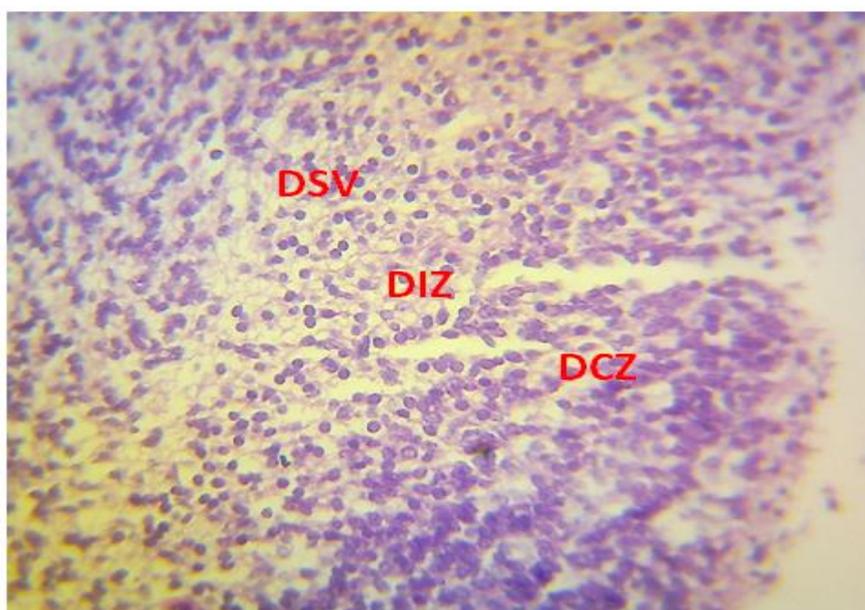
### Photomicrographs



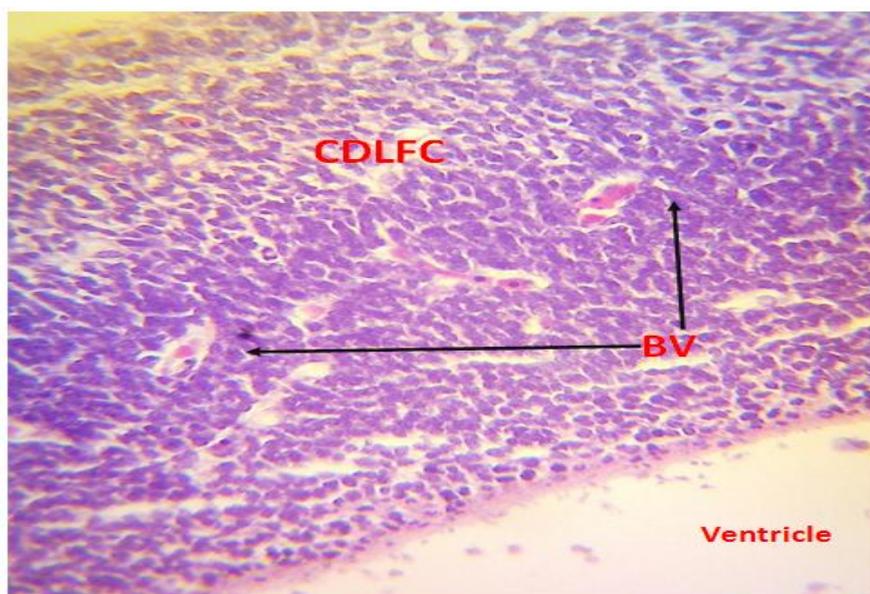
**PLATE 1: Photomicrographs (A & B) of control group showing normal histological architecture of the frontal cortex: Marginal Zone (MZ), Cortical Zone (CZ), Intermediate Zone (IZ), Subventricular Zone (SZ) and Ventricular Zone (VZ). Blood Vessels (BV) of the control group. Mag X 400 H&E.**



**PLATE 2:** Photomicrograph of the frontal cortex in low dose treated with 750mg/kg of *Aspilina africana* leaf extract showing layers of the developing frontal cortex (MZ, CZ, IZ, SV and VZ). Hyperplasia and Hypertrophy of subventricle zone (HHSZ), reduction in the Intermediate and Cortical Zones (RIZ and RCZ). Tiny blood vessels (TBV). Mag X 400 H&E.



**PLATE 3:** Photomicrograph of the frontal cortex in medium dose group treated with 1000mg/kg of *Aspilina africana* leaf extract showing a level of distortion; distorted cortical zone (DCZ), distorted cells of intermediate zone (DIZ) and distorted subventricular zone (DSZ). Mag X 400 H&E.



**PLATE 4: Photomicrographs of the frontal cortex in high dose group treated with 1250mg/kg of leaf extract of *Aspilia African* showing complete distortion of the layers of the frontal cortex (CDLFC) with blood vessels (BV) and numerous vacuolations. Mag X 400 (H&E).**

#### DISCUSSION

The frontal lobe plays a large role in voluntary movement. It houses the primary motor cortex which regulates activities like walking. The function of the frontal lobe involves the ability to project future consequences resulting from current actions, the choice between good and bad actions, the override and suppression of socially unacceptable responses and the determination of similarities and differences between things or events (Kimberg and Farah, 1993).

In this research work, the oral administration of the aqueous leaf extract of *Aspilia Africana* to pregnant Wistar rats on days 7-11 of gestation was found to alter the microanatomy of the developing foetal frontal cortex in Wistar rats. The various doses of the extract administered causes hyperplasia, hypertrophy, hypertrophy and slight to mild distortion of the layers of frontal cortex as seen in the histological sections. The effect being more on the medium dose and high dose groups that the mothers received 1000mg and 1250 mg of the extract per kilogram body weight especially on the cortical, intermediate and subventricular zones. The effects may be due to some of the phytochemical constituent of the *Aspilia africana* and the period of administration which may be teratogenic to developing frontal cortex thus altering their functions. The result obtained has clearly showed that the aqueous leaf extract was able to pass through the blood placenta barrier, which is the connection between the mother and the foetus to affect on the frontal cortex of the developing cerebral cortex.

The malformation of the nervous system in experimental animals have mostly been reported to occur only when the teratogen is administered at the critical period of the fetal development, mainly, shortly before or during the

closure of the neural groove (Singh et al, 1972). This sensitive period is the time of development during which the nervous system is highly susceptible to the effects of harmful internal and external conditions thereby causing various forms of damage to the system in the neonatal period (Ezurumlu and Killackey, 1982). Damage to the PFC has been found to affect decision making abilities and lead to impulsive behaviour (Bechara et al., 1994).

The result of the present study showed that the effect of *Aspilia africana* leaf extract on the fetal frontal cortex was dose-dependent as seen in the case of the low dose to that of high dose group. Ito et al., (1972) reported that the greater the severity of insults, the more rapid progression of cellular injury, the principle holds true for toxicological insults to the brain (Martins et al., 1984). The cellular distortion effect of *A. africana* leaf extract has also been reported by Eweka (2007 & 2006).

The result also showed that the the leaves extract have some level of effect on the blood vessels as blood vessels in the control group appeared much larger and clearly seen in almost all the five layers of the frontal cortex than in the treated groups however, Achonye (1976) reported the juice of the leaves of *A. africana* to be haemostatic and vasoconstrictive.

The increase in cellular hyperplasia/hypertrophy, especially in the low dose group and that of the high dose group may be as a result of cellular proliferation; a mechanism which is not yet clear as also reported by Eweka (2006). Adedayo and Adekilekun (2012) reported that administration of aqueous seed extract of *Datura stramonium* to wistar rats causes degenerative changes, vacuolations and progressive cell death of the cells within the frontal cortex and hippocampus of the treated rats when compared with the control group.

Fowler et al., (2014), using histological analysis combined with stereological technique, demonstrated that the prefrontal cortex (PFC) which is part of the frontal cortex is vulnerable to chronic, alcohol-induced oxidative stress and neuronal cell death. Excessive oxidative stress and subsequent DNA damage can be responsible for neuronal apoptosis and neuronal dysfunction associated with different neurological pathologies (Jacintho and Kovacic, 2002; Choi et al, 2012). The alkaloids present in *A. Africana* especially the alkaloids may have exerted their influence on the frontal cortex neurons causing axonal distortion and disarrangement of cells in the developing frontal cortex of the foetuses. Maryam et al. (2013) showed in their work that Vinca alkaloid, which has medicinal value for the treatment of certain diseases including the treatment regimens for testicular carcinoma, that alkaloid was found to be neurotoxic at higher doses.

### CONCLUSION

This research work shows that the consumption of *A. africana* leave extract have adverse effect on the histology of the frontal cortex of the developing foetus of rat. This histological alteration can affect the functions of the frontal cortex.

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