Niger. J. Physiol. Sci. 36 (December 2021): 237– 240
www.njps.physiologicalsociety.com
https://doi.org/10.54548/njps.v36i2.13

Niger. J. Physiol. Sci.

Short Communication

Teratogenic Effect of Aqueous Leaf Extract of *Aspilia africana* on The Dentate Gyrus of Wistar Rat Fetuses

***Asuquo, O.R[1]., Edet, P.E[1]., Eluwa, M.A[1]., Kennedy, O.O.O[2]**

[1]*Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.*
[2]*Department of Animal Sciences, Faculty of Agriculture and Wildlife Resources Management, University of Calabar, Calabar, Nigeria*

Summary: *Aspilia africana* is an herbal plant widespread in Africa used for medicinal purposes and also used by pregnant women for health-related issues. This study was aimed at investigating the teratogenic effect of aqueous leaf extract of *Aspilia africana* on the dentate gyrus of Wistar rat fetuses. Twenty (20) female adult rats weighing between 190-205g were used for this study. The rats were divided into four groups; control, low dose, medium dose and high dose with each group containing five rats. Pregnancy was induced by caging the female rats with sexually matured males. The presence of vaginal plug and tail structure in the vaginal smear the following morning confirmed coition, and it was regarded as day 0 of pregnancy. The control group was given distilled water. The low dose, medium dose, and the high dose groups received 750mg/kg, 1000mg/kg, and 1250mg/kg body weight of aqueous leaf extract of *Aspilia africana* through an orogastric tube from day 7-11 of gestation. On the 20th day of gestation, the animals were sacrificed using chloroform-inhalation method. Their fetuses were harvested via uterectomy, the brain was excised and fixed in 10% buffered formalin, and then routine histological processes were carried out. Staining was done using Haematoxylin and Eosin method, histometric measurements were measured**.** Histological observation of the dentate gyri of experimental groups revealed marked distortion, reduction of the polymorphic layer, hyperplasia and hypertrophy of cells in the molecular and granular layer especially in the high dose group whose mothers received 1250mg/kg of the extracts. Histometric analysis revealed reduction of cell diameter, total number of cells in the square of pyramidal cells and packed density of cells especially in the group whose mothers were treated with high dose of plant extract. The result suggests high doses of aqueous leaf extract of *Aspilia africana* may be teratogenic to the dentate gyrus of Wistar rat fetuses.

Keywords: *Dentate gyrus, Hyperplasia, Hypertrophy, Neurogenesis, Memory loss, Teratogenic*

*Address for correspondence: *ola_asuquo@yahoo.com; Tel: +232-816-298-9121*

Manuscript received- July 2021; Accepted- November 2021

INTRODUCTION

The dentate gyrus is an organ found in the brain which is responsible for learning and formation of new memories. It takes part in the relay of synaptic transmission in the hippocampus and is known to take part in the formation of new episodic memories (Amaral *et al*, 2007; Saab *et al*, 2009), spontaneous exploration of novel environments (Saab *et al*, 2009) and other several functions (Scharfman, 2007). It is one of the few regions of the adult brain where adult neurogenesis takes place in many species of mammals (Cameron and McKay, 2001; Piatti *et al*, 2013; Benarroch, 2013; Brickman *et al*, 2014; Zhang and Zhang, 2018). Neurogenesis is thought to play a role in the formation of new memories (Kitabatake *et al*, 2007; Deng *et al*, 2010). According to Nakashiba *et al* (2012), new memories could preferentially use newly formed dentate gyrus cells, providing a potential mechanism for distinguishing multiple instances of similar events or multiple visits to the same location. Several researches have been carried out on effect of some plants on the dentate gyrus (Jahanshashi *et al*, 2007; Marzban *et al*, 2011). 70-80% of Africa's population relying on traditional medicines, the importance of the role of medicinal plants in the healthcare system cannot be ruled out (Mander and Breton, 2006). Nigeria has a great variety of natural vegetation which is used in trado-medicine to cure various ailments. In Nigeria, *Aspilia africana* is known by different names like "edemedong" in Efik and Ibibio, "orangila" in Igbo, "yunyun" in Yoruba, and "toozalin- yan-maata in Hausa (Single, 1965). *Aspilia Africana* has been shown to exhibit a wide range of biological activities including antiviral, fungicide and antibacterial activities and for the treatment of several ailments such as gonorrhea, tuberculosis, cough, rheumatic pains, stomach trouble, corneal opacity, wounds healing (Iwu, 1993; Okoli *et al*, 2007; Okwuonu *et al*, 2008; Christian *et al*, 2012; Komakech *et al*, 2019)

The dentate gyrus is of particular interest as new dentate granule cells (GCs) are generated continuously in the adult mammalian brain (Cameron and McKay, 2001; Ming and Song, 2011). Impairment of the dentate gyrus leads to memory loss as information cannot be consolidated into the working memory. It also leads to stress and depression due to increase in neurogenesis in the dentate gyrus which leads to physiological effects of stress and depression. There is paucity of literature on the teratogenic effect of aqueous extract of *Asipilia africana* on the dentate gyrus of albino rat fetuses, hence the study.

MATERIALS AND METHODS

Breeding of Animals: Twenty (20) adult Wistar rats of weighing between 180-200g were used for the research work. The rats were obtained from the Faculty of Basic Medical Sciences animal house, University of Calabar, Calabar. They were kept in standard wooden cages with iron nettings and kept in the animal house of the Department of Anatomical Sciences, Faculty of Basic Medical science, University of Calabar, Calabar, Nigeria. The animals were randomly divided into four groups and were kept in a stable and standard environmental condition facilitated by proper ventilation and approximate room temperature (of about 25-27ºc photo period 12 hours natural light. 12 hours dark and humidity 45-50%) throughout the duration of the experiment. The animals were fed daily and regularly with vital feed (grower mesh) obtained from Vital Food Company No: 44 Nelson Mandela Street Calabar and water were given *ad libitum*. Cleaning and replacement of beddings were done regularly to maintain a good hygienic status for the animals.

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.

Preparation of *Aspilia africana* Leaf Extract: 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 by a botanist 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. It was filtered first with sieve basket and the chess cloth and later Whatman number one filter paper. The filtrate was concentrated in a water bath which was regulated to 50ºc and yielded a thick-semi solid paste of brown colour which is the crude extract. The extract was stored in a refrigerator from where it was taken from for oral administration.

Experimental Protocol: The animals were randomly selected and divided into four groups labelled control, low dose, medium dose and high dose, with each group consisting of five (5) rats. The oestrous cycle of the animals was checked with normal saline to be sure that they were due for mating. Each female rat's oestrous cycle was determined by daily vaginal lavages and at oestrous, each rat was caged overnight with a sexually active male rat of the same strain. The morning after a vaginal smear showed the presence of sperm in the female tract, the process of spermatozoa signified 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/kg of the aqueous extract of *Aspilia africana* per body weight.

Medium Dose: The rats were administered with 1000mg/kg of the aqueous extract of *Aspilia africana* per body weight.

High Dose: The rats were administered with 1250mg/kg of the aqueous extract of *Aspilia africana* per body weight.

The extract was administered to each animal in the experimental group based on their body weight and administration was done using the oral route through orogastric intubation from days 7-11 of gestation respectively. The rats were sacrificed on the 20th day of pregnancy using chloroform inhalation method. The fetuses of each group were collected after opening the anterior abdominal wall, fixed and then processed for histological studies. Histometric measurements were measured such as diameter of cells, total number of cells in the square and packed density of cells.

Diameter of Cells: The diameter of the pyramidal cells of the dentate gyrus were calculated using the formula;

Diameter of a cell= Axial ratio X calibration constant

Total number of cells in the square: Stained sections were focused under 40X magnification objective lens of the microscope. Counting was done under 400X magnification. The pyramidal cells of the dentate gyrus were counted. The cell region for calculation was selected using random selection technique from the serial section from each group.

Packed density of cells: The packed cell density was calculated using the method of Ramar and Sarawathi (2013).

RESULTS

Table 1 shows the histometric results of the diameter of cells, total number of cells in a square and packed density of cells in the control and experimental dentate gyrus of fetuses. There was a significant reduction in the parameters measured in the experimental groups when compared to control especially in high dose group fetuses whose mothers were treated with 1250mg/kg body weight of extract.

Table 1:
Histometric characteristics of the fetal dentate gyrus in the various groups

Groups	Diameter of cells (µ)	Total number of cells in the square	Packed density of cells per square (x10cubic/mm
Control	3.98±0.16	982.40±24.50	128.52±5.25
Low dose	3.20±0.18	760.50±12.40	104.83±6.27
Medium dose	2.94±0.29[a]	698.50±17.5[a]	96.35±8.25[a]
High dose	2.48±0.27[b]	486.28±10.6[b]	82.64±8.06[b]

Values are mean ± SEM (n=5)

[a] Significantly different from control at p≤0.05 b- significantly different from control at p≤0.01

.

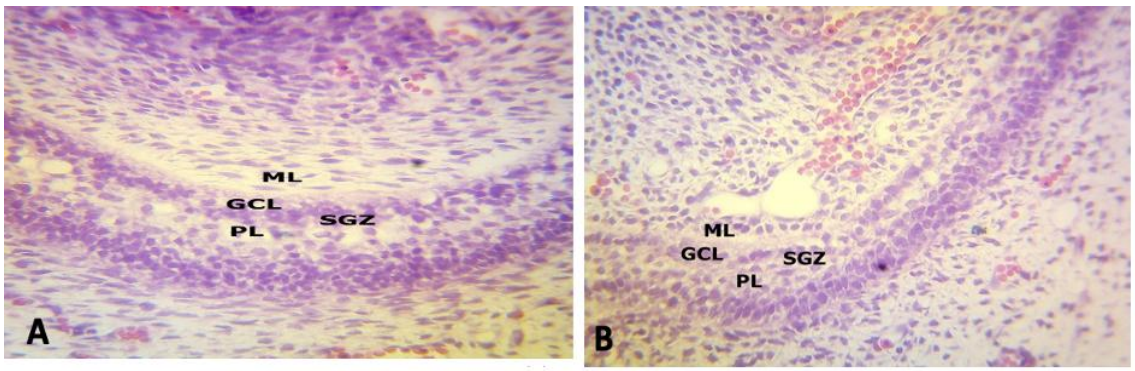

Plate 1:
Photomicrograph of the dentate gyrus of control and low dose group which received 750mg/kg 0f aqueous leaf extract of *Aspilia africana* (H & E × 400 for all plates).
A. Control dentate gyrus showing its three distinct layers; molecular layer (ML), polymorphic layer (PL), and granular layer (GL) with the subgranular zone (SGZ).
A. Dentate gyrus of low dose group showing the molecular layer (ML), polymorphic layer (PL), and granular layer (GL) and the subgranular zone (SGZ) with marked distortion and reduction of the polymorphic layer (PL), slight hypertrophy of cells in the molecular layer (ML) and distortion of cells in the granular layer (GL).

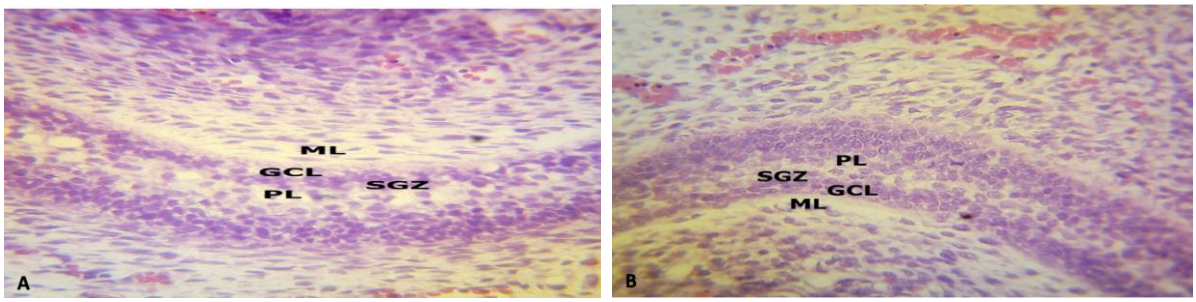

Plate 2:
Photomicrograph of the dentate gyrus of control and medium dose group which received 1000mg/kg 0f aqueous leaf extract of *Aspilia africana* (H & E × 400 for all plates).
A. Control dentate gyrus showing its three distinct layers; molecular layer (ML), polymorphic layer (PL), and granular layer (GL) with the subgranular zone (SGZ).
A. Dentate gyrus of low dose group showing the molecular layer (ML), polymorphic layer (PL), and granular layer (GL) and the subgranular zone (SGZ) with marked distortion and reduction of the polymorphic layer (PL), slight hypertrophy of cells in the molecular layer (ML) and distortion of cells in the granular layer (GL).

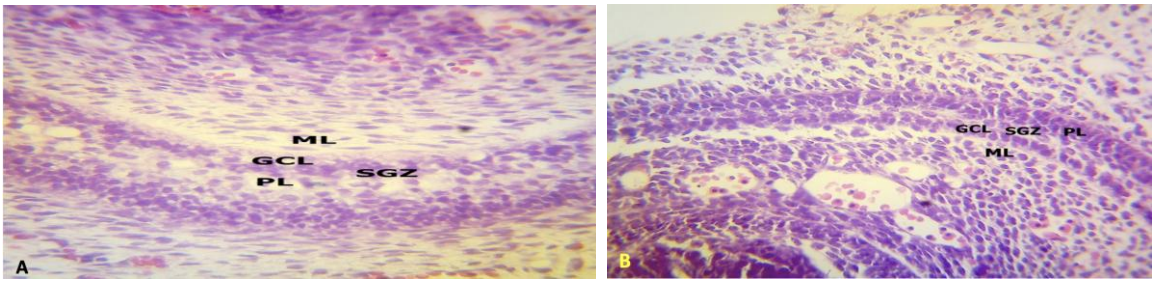

Plate 3: Photomicrograph of the dentate gyrus of control and high dose group which received 1250mg/kg 0f aqueous leaf extract of *Aspilia africana* (H & E × 400 for all plates).
A. Control dentate gyrus showing its three distinct layers; molecular layer (ML), polymorphic layer (PL), and granular layer (GL) with the subgranular zone (SGZ).
A. Dentate gyrus of low dose group showing the molecular layer (ML), polymorphic layer (PL), and granular layer (GL) and the subgranular zone (SGZ) with marked distortion and reduction of the polymorphic layer (PL), slight hypertrophy of cells in the molecular layer (ML) and distortion of cells in the granular layer (GL).

DISCUSSION

Medicinal plants are the bedrock of primary health care for majority of world's population since the beginning of civilization (Pan *et al*, 2014). The dentate gyrus is an organ found in the brain responsible for learning and formation of new memories and composed of unidirectional projections dispersed towards CA3 pyramidal cells of the hippocampus (Treves *et al*, 2008). In rats, approximately 85% of the granule cells are generated after birth (Bayer, 1974); while, in humans, it is estimated that granule cells begin to be generated during gestation weeks 10.5 to 11, and continue being generated during the second and third trimesters after birth and all the way into adulthood (Bayer *et al*, 1982; Eriksson *et al*, 1998; Bayer *et al*, 2008). Studies have shown that after destroying about 90% of their dentate gyrus cells, rats had extreme difficulty in maneuvering through a maze they had been through, prior to the lesion being made. When

being tested a number of times to see whether they could learn a maze, the results showed that the rats did not improve at all, indicating that their working memories were severely impaired. Rats had trouble with place strategies because they could not consolidate learned information about a maze into their working memory, and, thus, could not remember it when maneuvering through the same maze in a later trial.

Every time a rat entered the maze, the rat behaved as if it was seeing the maze for the first time (Xavier 2009). From this study, it has been shown that aqueous leaf extract of *Aspilia africana* affects the dentate gyrus by causing hyperplasia, hypertrophy and distortion of the molecular, polymorphic and granular layers which received 750mg/kg, 1000mg/kg and 1250mg/kg body weight of the extract. The effect was dose dependent and may have been due to the effect of the chemical constituent of the extract. The teratogenicity may affect learning and formation of new memories.

REFERENCES

Amaral D.G., Scharfman, H.E., Lavenex, P (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). Prog. Brain Res, 163: 3-22.

Bayer S.A and Altman J (2008). The human brain during the early first trimester. 5 Atlas of Human Central Nervous System Development. Appendix, p. 497.

Bayer, S. A., Yackel, J. W., Puri, P. S (1982). Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. Science. 216 (4548): 890–892.

Bayer, S. and Altman, J (1974). Hippocampal development in the rat: cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation. J Compar Neurol. 158 (1): 55–79.

Benarroch E.E (2013). Adult neurogenesis in the dentate gyrus: general concepts and potential implications. Neurol 81(16): 1443-1452.

Brickman A.M., Khan, U.A., Provenzano, F.A., Yeung, L.K., Suzuki, W., Schroter, H., Wall, M, Sloan, R.P and Small, S.A (2014). Enhancing dentate gyrus function with dieting flavonols improves cognition in older adults. Nat Neurosci 17(12): 1798-1803.

Cameron, H. A., and McKay, R. D (2001). Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Compar Neurol. 435, 406–417.

Christian A.G., Mfon, A.G., Dick, E.A., Oku, E.D., Linus, A.J and Chukuma, E.B (2012). Antimalarial potency of the leaf of *Aspilia africana* (Pers.) C.D.Adams. Asian Pac J Trop Med 5(2): 126-129.

Deng, W., Aimone, J. B., and Gage, F. H (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nature Rev Neurosci 11 (5): 339–350.

Eriksson, P S., Perfilieva, E., Björk-Eriksson, T. Ann-Marrie, A., Claes Nordbeorg, Daniel A., and Fred H. G (1998). Neurogenesis in the adult human hippocampus. J Nat Med. 4 (11): 1313–1317.

Iwu M. M (1993). Handbook of African Medical Plants. Boca Raton, FL: CRC press; Pp 367.

Jahanshahi, M., Sadeghi, Y., Hosseini, A., Naghdi, N (2007). The effect of spatial learning on the number of astrocytes in rats' dentate gyrus. Neuroanat. 6:51–53.

Kitabatake, Y., Sailor, K.A., Ming, G and Song, H (2007). Adult neurogenesis and hippocampal memory function: new cells, more plasticity, new memories? Neurosurg Clin N Am 18(1): 105-113.

Komakech, R., Matsabisa, M.G and Kang, Y (2019). The wound healing potential of *Aspilia africana* (Pers.) C.D. Adams (*Asteraceae*). Evid Based Complement Alternat Med. 7957860. 1-12.

Mander, M. and Breton, G. (2006). Overview of the Medicinal Plants Industry in Southern Africa. In: Commercialising Medicinal Plants in Southern Africa Guide, Diederichs, N. (Ed.). Sun Press, South Africa, p: 3-8.

Marzban, M., Asi, S.S., Huseini, H.F., Tondar, M., Choopani, S., Mehdizadeh M (2011). Effects of butternut squash extract on dentate gyrus cell proliferation and spatial learning in male adult rats. Trad Chinese Med neurodegen 692): 1855-1859.

Ming, G. L., and Song, H (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70, 687–702.

Nakashiba, T.; Cushman, J. D.; Pelkey, K. A.; Renaudineau, S.; Buhl, D. L.; McHugh, T. J.; Rodriguez Barrera, V.R., Chittajallu, R., Iwamoto, K.S., McBain, C.J., Fanselow, M.S., Tonegawa, S (2012). Young dentate granule cells mediate pattern separation whereas old granule cells facilitate pattern completion. Cell, 149(1): 188-201.

Okoli, C. O., Akah, P. A and Okoli, A (2007). Potential of leaves of *Aspilia africana* (Compositae) in wound care: an experimental evaluation. BMC Complement Altern Med, 7: 24.

Okwuonu, C. U., Oluyemi, K. A., Baxter, G. D., Adesanya, O. A., Ukwenya, V. O., Odion, B.I., and Ofusori, D. A (2008). Effects of Methanolic Extract of Aspilia africana leaf on the ovarian tissues and weights of wistar rats. Internet J Altern Med 5(2).

Pan, S.Y., Litscher, G., Gao, S.H., Zhou, S.F., Yu, Z.L., Chen, H.Q., Zhang, S.F., Tang, M.K., Sun, J.N and Ko, K.M (2014). Historical perspective of traditional indigenous medical practices: The current renaissance and conversation of herbal resources. Evidence-based Complem Alt Med. 525340, 1-20.

Piatti, V.C., Ewell, L.A and Leutgeb, J.K (2013). Neurogenesis in the dentate gyrus: carrying the message or dictating the tone. Front. Neurosci. 7: 50.

Ramar, S and Saraswathi, P (2013). Histomorphometric study of *fructus psoralcea* in ethanol induced neurodegeneration of hippocampus in rats. J. Clin. Diagn. Res 7(8): 1561-1564

Saab B.J., Georgion, J., Nath, A., Lee, F.J., Wang, M., Michalon, A., Liu, F., Mansry, I.M., Roder, J.C (2009). NCS-1 in the dentate gyrus promotes exploration, synaptic plasticity, and rapid acquisition of spatial memory. Neuron. 63(5): 643-656.

Scharfman, H.E (2007). The dentate gyrus: A comprehensive guide to structure, function and clinical implications. Prog. Brain. Res. 163: 1-823.

Single, S. C (1965). Medicinal Plants of Nigeria, 1st Edition. Nigerian National Press, Apapa. P: 31-34.

Treves A., Tashiro, A., Witter, M.P., Moser, E.I (2008). What is the mammalian dentate gyrus good for? *Neuroscience* 154*:* 1155–1172.

Xavier, G. F (2009). Dentate gyrus and spatial behavior. Prog Neuro-Psychopharmacol Biol Psychiatry. 33 (5): 762–773.

Zhang, L and Zhang, X (2018). Factors regulating neurogenesis in the adult dentate gyrus, the hippocampus- plasticity and functions. Intechopen, 75631