



**ISN**  
International Society  
for Neurochemistry

**Committee for Aid and Education in Neurochemistry (CAEN)**

**CATEGORY 1B: Research supplies for use in the applicant's home laboratory**

**Report by AYANNUGA Olugbenga Ayodeji**

**KAOLIN-INDUCED HYDROCEPHALUS IN JUVENILE RATS; CEREBRAL PROTECTION  
BY ETHANOL EXTRACT OF *Juglans regia* L NUTS.**

**Introduction**

Hydrocephalus remains a debilitating condition with unwanted sequelae particularly in cases of delayed surgical intervention (Del Bigio, 2004; Crews et al., 2004). Since the 1950s, following the advent of silicone shunts, surgical intervention remains the only known remedy for the anomaly (Richards et al., 2009; Akalan, 2011; Srinivasan et al., 2014). Delay in the surgical management of hydrocephalus usually results in neurological deficit which had been severally attributed to oxidative stress and inflammatory processes resulting in progressive neuronal and glial degeneration (Socci et al., 1999; Cao et al., 2012; Damkier et al., 2013). In economically disadvantaged nations of the world, lack of fund and sparse infrastructure and manpower regularly results in such delays (Warf, 2010). Such delays are made worse by culturally-driven parental denials that follow the diagnosis of hydrocephalus, this eventually result is neurological deficiencies occasioned by structural anomalies of myelin and axon (Ayannuga et al., 2015).

*Juglans regia* L (Walnut) is a well-known nut that is traditionally consumed in several parts of the world. It is known to contain several neuroprotective

substances such as melatonin, folate and vitamin E (Reiter et al., 2005). It has the highest concentration of antioxidants amongst all nut and among the first two of dietary plants (Halvorsen et al., 2002; Pellegrini et al., 2006). Aside from its antioxidative capacity, it is also known to have anti-inflammatory properties by significant reduction of nitric oxide (NO) generation (Willis et al., 2010; Jin et al., 2016). The polyphenol-rich extract of walnut has been reported to protect low density lipoprotein from oxidative damage (Anderson et al., 2001). The possible protective effect of polyphenol-rich extract of walnut with the aim of serving as a pre-surgical therapy in hydrocephalus has not been properly explored.

### **Specific aims**

The aim of this work was to determine the possible protective effect of ethanol extract of walnut as a pre-operative intervention for the management of obstructive hydrocephalus, particularly in the protection of cellular and structural components of the cerebral cortex of juvenile rats following kaolin-induced hydrocephalus.

### **Methodology**

Ethical approval for this study was obtained from the Health Research Ethical Committee of the College of Health Sciences, Obafemi Awolowo University, Nigeria. Rats were handled in accordance with the principles of laboratory animal care (National Institutes of Health (NIH) publication No. 85-23, revised 1985). Sixty 3 weeks old rats were raised in the animal holding of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Nigeria. The juvenile rats were randomly divided into 3 groups of 20 rats each. Group A rats were excluded from kaolin suspension injection and served as the control group. Groups B and C rats

were induced by percutaneous injection of 200mg/ml kaolin suspension into the cisterna magnum under anesthesia.

*Juglans regia* nuts (Walnut) were obtained from the local market and authenticated at the Botany Department of Obafemi Awolowo University, Nigeria. The walnut seed was grounded into powder using manual grinding machine. The grounded material (200 g) was defatted with 2 liters of n-hexane, stirred for 24 hours using magnetic stirrer and filtered. The residue was air-dried at room temperature. The dried residue was extracted using 2 liters of ethanol, stirred continuously for 24 hours, followed by filtration. Filtrations were done with Whatmann's paper 11. The extraction process was repeated with fresh ethanol and filtrate was combined. The combined filtrate was concentrated in vacuo at 45 °C in a rotary evaporator. The filtrate was subsequently kept in a desiccator until time of use.

Groups C rats received 50 mg per kilogram body weight of walnut filtrate (WF) suspended in normal saline on daily basis. WF was administered orally by an oral cannula for 14 days to 10 rats in groups B. WF administration commenced 24 hours after induction of hydrocephalus. Rats in group B were administered normal saline.

Rats in all the groups were subjected to neurobehavioral test of memory and cognition using Morris water maze before induction and twice weekly subsequently. Ten rats in each group were sacrificed by cervical dislocation at the completion of the 14 days of WF administration. The brains were harvested on ice and fixed in Neutral buffered formalin. Brain slices obtained at the level of the optic chiasma were processed for histology, stained with Haematoxylin and Eosin and Luxol fast Blue stain.

## **Result**

The study revealed a significant reduction in density of cortical neurons following hydrocephalus, which was reversed by the concomitant ingestion of the walnut extract. A significant increase in degenerating neuron was noted in the hydrocephalic rats, however there was no significant difference in the density of degenerating neuron between the non-hydrocephalic rats and the hydrocephalic rats that were administered the walnut extract. This pattern was noted in the upper and middle cortical regions comprising of cortical layers I/II and layers III/IV respectively. Though the differences in the density of degenerating neurons was as stated above in the inner cortical region (consisting of cortical layers V/VI), there was no significant differences in the density of normal neurons in this region.

Densities of normal and degenerating oligodendrocytes across the 3 cortical regions were not significantly different across the control, hydrocephalic and hydrocephalic treated rats. In addition, the thickness and integrity of the subcortical white matter bundle across the 3 groups did not show any differences.

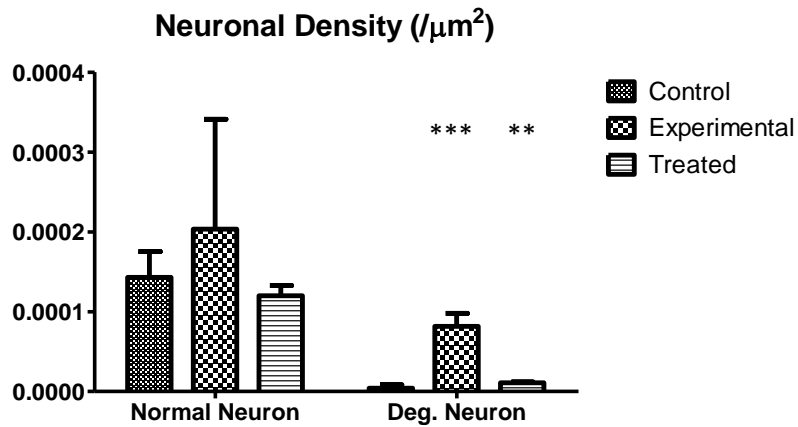
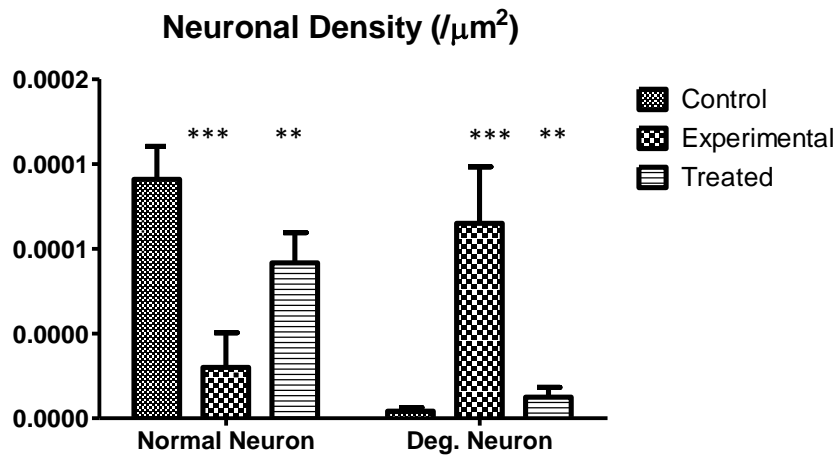
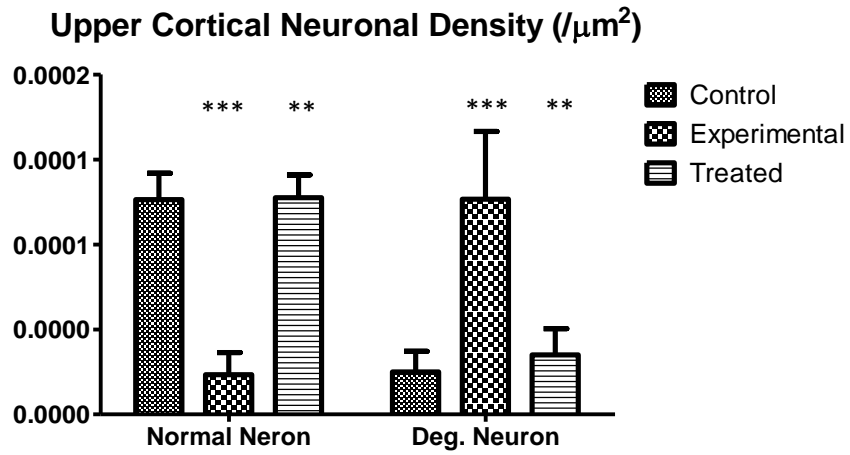


Fig. 1: Bar Charts showing the Neuronal density (Normal and Degenerating Neurons) in the upper, middle and inner cortical regions (upper, middle and lower charts respectively). \*\*\* Significant difference compared with control, \*\* Significant difference compared with Experimental.

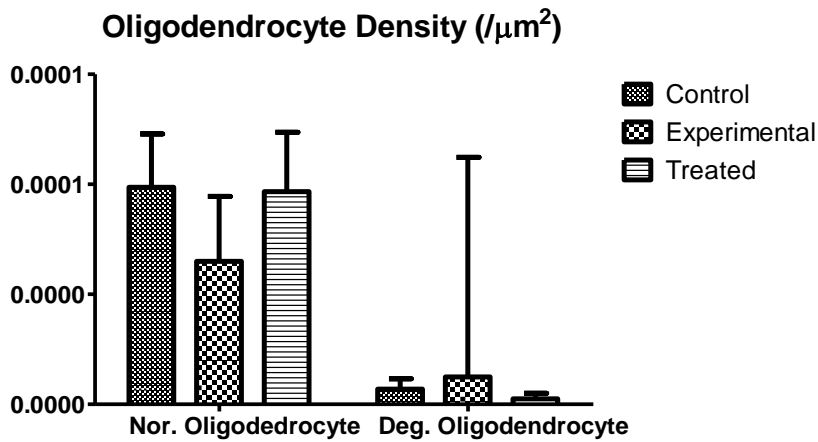
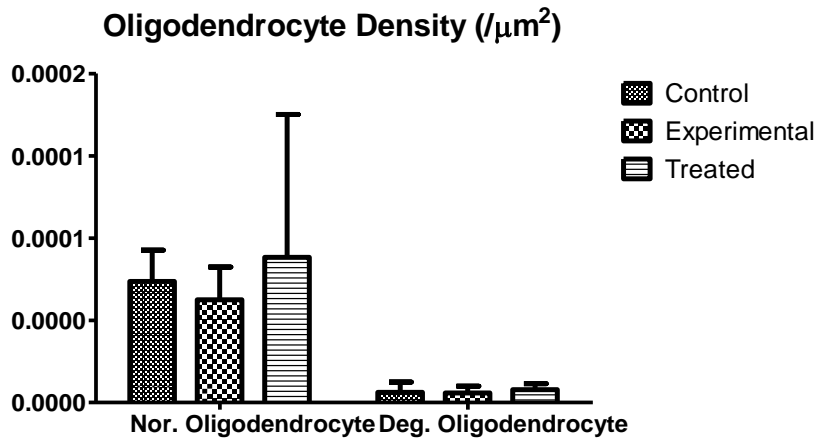


Fig. 2: Bar Charts showing the oligodendrocyte density (Normal and Degenerating Oligodendrocyte) in the upper, middle and inner cortical regions (upper, middle and lower charts respectively). Differences across groups in the normal and degenerating oligodendrocyte not significant.

## **Selected References**

1. Damkier HH, Brown PD, Praetorius J. Cerebrospinal fluid secretion by the choroid plexus. *Physiol Rev.* 2013; 93(4):1847–92.
2. Warf: Hydrocephalus treatment and outcome in African infants with myelomeningocele: what we have learned so far. *Cerebrospinal Fluid Research* 2010;7;(Suppl 1):S3.
3. Anderson, K.J.; Teuber, S.S.; Gobeille, A.; Cremin, P.; Waterhouse, A.L.; Steinberg, F.M. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. *J. Nutr.* 2001, 131, 2837–2842.
4. Willis, L.M.; Bielinski, D.F.; Fisher, D.R.; Matthan, N.R.; Joseph, J.A. Walnut extract inhibits LPS-induced activation of BV-2 microglia via internalization of TLR4: Possible involvement of phospholipase D2. *Inflammation* 2010, 33, 325–333.
5. Park, G.; Kim, H.G.; Hong, S.P.; Kim, S.Y.; Oh, M.S. Walnuts (seeds of *Juglandis sinensis* L.) protect human epidermal keratinocytes against UVB-induced mitochondria-mediated apoptosis through upregulation of ROS elimination pathways. *Skin Pharmacol. Physiol.* 2014, 27, 132–140.
6. Del Bigio, M.R. 2004. Cellular damage and prevention in childhood hydrocephalus. *Brain Pathology* 14:317-324.
7. Crews, L. Wyss-Coray, T. and Masliah, E. 2004. Insights into the pathogenesis of hydrocephalus from transgenic and experimental animal models. *Brain Pathology* 14.(3):312-6.
8. Cao, G. Gu, M. Zhu, M. Gao, J. Yin, Y. Marshall, C. Xiao, M. Ding, J. and Miao, D. 2012. Bmi-1 absence causes premature brain degeneration. *PLoS One* 7.(2):e32015.

9. Richards, H.K. Seeley, H.M. and Pickard, J.D. 2009. Efficacy of antibiotic-impregnated shunt catheters in reducing shunt infection: data from the United Kingdom Shunt Registry. *Journal of Neurosurgery-pediatrics* 4.(4):389-93.
10. Akalan, N. 2011. Myelomeningocele (open spina bifida) - surgical management. *Advances and Technical Standards in Neurosurgery* 37:113-41.
11. Socci, D.J. Bjugstad, K.B. Jones, H.C. Pattisapu, J.V. Arendash, G.W. 1999. Evidence that oxidative stress is associated with the pathophysiology of inherited hydrocephalus in the H-Tx rat model. *Experimental Neurology* 155.(1):109-17.
12. Reiter, R. J., Manchester, L., & Tan, D. X. (2005). Melatonin in walnuts: influence on levels of melatonin and total antioxidant capacity of blood. *Nutrition*, 21(9), 920-924.
13. Pellegrini, N., Serafini, M., Salvatore, S., Del Rio, D., Bianchi, M., & Brighenti, F. (2006). Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different in vitro assays. *Molecular Nutrition & Food Research*, 50(11), 1030-1038.
14. Halvorsen, B. L., Holte, K., Myhrstad, M. C., Barikmo, I., Hvattum, E., Remberg, S. F., Blomhoff, R., et al. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition* 132(3), 461-471.
15. Ayannuga O.A, Shokunbi M.T, Naicker T.A. Myelin sheath injury in kaolin-induced hydrocephalus: a light and electron microscopy study. *Pediatric Neurosurgery* 2016;51:61-68.
16. Jin Gyu Choi, Gunhyuk Park, Hyo Geun Kim, Dal-Seok Oh, Hocheol Kim and Myung Sook Oh In Vitro and in Vivo Neuroprotective Effects of Walnut (*Juglandis Semen*) in Models of Parkinson's Disease. *Int. J. Mol. Sci.* 2016, 17, 108.



## Finances

	Amount (USD)
<b>Award amount</b>	<b>4,965</b>
<b>Expenses</b>	
1. Animals (Purchase, Feeding and Housing)	670
3. Vortex Mixer and shaker	325
4. Magnetic stirrer	225
5. Microtome, Microtome Blade with adaptable Blade holder	2,490
6. Glass wares	530
7. Micropipettes	225
8. Histology Consumables and Stain	420
9. Kaolin Powder	70
<b>Total Expenses</b>	<b>4,955</b>
<b>Balance</b>	<b>10</b>

The CAEN award has been very helpful in setting up of a neurobiology laboratory, particularly in getting the above mentioned equipment/materials for my personal use as a researcher in the laboratory as well as the use of my graduate students. A manuscript is been prepared for publication from the project undertaken with the grant.

