Int J Pharm Bio Sci 2012 July; 3(3): B 118 - 125

Research Article **Anatomy**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

SOME MICROHISTOLOGICAL CHANGES IN SUPERIOR COLLICULUS FOLLOWING CHRONIC INTAKE OF AQUEOUS NEEM LEAVES EXTRACT IN ADULT WISTAR RATS (*RATTUS NORVEGICUS*)

FAKUNLE P.B AND AJIBADE A.J

Anatomy Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

ABSTRACT

The plant, *Azadirachta indica* A Juss, family Meliaceae that is commonly used for the traditional treatment of malaria and other associated conditions in form of decoction, in which unspecified quantities are usually consumed without due regards to toxicological and other adverse effects .The present study was designed to investigate some effects of chronic *Azadirachta indica* (Neem) leaf extract on the microarchitecture of the visual relay center superior colliculus of adult wistar rats the relationship of which scanty literature is available as at present. Forty adult wistar rats both sexes of average weight 190±21.6g were randomly distributed after acclimatization into 4 groups (n=10) T1,T2, T3 and C such that rats in groups T1,T2 and T3 were given 400mg/Kg.B.wt,300mg/Kg.B.wt and 200mg/Kg.B.wt of aqueous neem extract while group C (control) received distilled water all for 14 days after which the rats were sacrificed by whole body intracardiac perfusion fixation and processed for routine histological techniques.The respective slides were then stained for Nissls substance. Statistical analysis showed insignificantly decreased $P<0.05$ weight (mean± sem, 202.4±28.3,207±9.6&213± 3.24) g in groups T1, T2 and T3 respectively compared to the control group with mean± sem, 217.4±7.3g. Significantly reduced neuronal density ($P>0.05$) of 44% and 38% neuronal loss in treatment groups T1 and T2 compared to control group was recorded. Histological findings showed distorted pyramidal neurons with scanty distribution of glial cells more marked in group T1 but less pronounced in group T2 while sections in T3 and Control groups appear normal. These alterations may affect normal functionality of superior colliculus most especially in the maintenance of saccade

KEYWORDS : Superior colliculus, Neem, Pyramidal neurons, Saccade, Vision

FAKUNLE P.B

Anatomy Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

***Corresponding author**

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization (Nayak Aarati et al, 2011). *Azadirachta indica*, also known as neem, belongs to the family of meliaceae (Yanpallewar et al., 2003). It is one of the most useful medicinal plants (Kausik and Ranajet, 2002). Each part of the neem tree has some medicinal property and is thus commercially exploitable. During the last five decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.(Kausik, et al, 2007).Malaria fever being a global deadly disease most especially over the coast of Africa has being more lethal due poverty and people tend to take solace in herbs of various forms as neem leaf extract has been prescribed for oral use concerning treatment of malaria. Dried neem leaves in the form of tea are used by the people of Nigeria and Haiti to treat this disease (Fujiwara, *et al,* 1984).However over indulgence in the use of this drug which has always culminated into chronic consumption has being a problem of public health concern most especially as it affects some very vital organs including the brain bearing the superior colliculus involved in various types of sensori-motor processing (King 2004). Among these functions, orienting behaviors are known to be controlled by a group of output neurons of its intermediate gray layer that send descending projections to the contralateral brain stem gaze center in the medial pontine reticular formation (MPRF) (Grantyn and Berthoz 1985; Isa and Sasaki 2002). Understanding the mechanisms responsible for the translation of sensory signals into the commands for movement is one of the fundamental goals of neurobiology. Orientation of the eyes toward external stimuli is a common choice for analyzing these mechanisms not only because it is a nearly universal behavior but also because the constant relationship between stimulus location and the direction of a gaze shift suggests that superior colliculus may provide a relatively simple model for studying how sensory systems initiate and guide movement. In the superior colliculus, the juxtaposition of cellular layers with sensory and motor functions is especially useful for studies of these mechanisms Although behavioral studies have shown that superior colliculus is not needed for object recognition, but plays a critical role in the ability to direct behaviors toward specific objects, and can support this ability even in the absence of the cerebral cortex concerned (Sparks,1999).The superior colliculus also provides an ample opportunity to investigate how, within a single brain structure, signals from the different senses are combined and used to guide adaptive motor responses (Andrew, 2004) We believe our investigation of chronic intake of the aqueous neem leaves extract will further improve our understanding of the cellular integrity of superior colliculus when assaulted by chronic consumption of aqueous extract of azadirachta indica leaves.

MATERIALS AND METHODS

Fourty adult wistar rats (both sexes) of average weight 190±21.6g were bought from the Animal Holdings in the Anatomy Department of Obafemi Awolowo University, Ile-Ife, Nigeria. They were carefully assessed, screened and confirmed to be free from any pathological conditions. The rats were maintained in the Animal House of the Department of Anatomy Ladoke Akintola University of Technology Ogbomosho, Nigeria, and fed with standard mouse chow (Ladokun Feeds, Ibadan Nigeria) with water, *ad libitum* for an acclimatization period of two weeks during which their weight and feeding

patterns were carefully monitored and documented. After acclimatization the rats were randomly assigned into four groups (N=10) of (Treatments T1 T2 & T3 and control C). Daily weights were taken and documented.Fresh Neem leaves were picked in large quantity from Neem tree in the Biological garden of the Biology department of Ladoke Akintola University of Technology, Ogbomoso. The leaves were air dried in the Microbiology department for a period of 2weeks. The dried neem leaves were then grinded into a fine powdery form and then taken to the Food sciences department of Ladoke Akintola University of Technology, Ogbomoso, where the crude aqueous extract was obtained. Administration was done orally with the oral cannula. Group T1 animals were given 400mg/Kg.Bwt, Group T2 animals were given 300mg/Kg.Bwt while Group T3 animals were given 200mg/Kg.Bwt. The control animals were given distilled water. The administration was allowed to proceed for a period of 14 days. Changes in body weights were documented.At the end of administration period, the rats were sacrificed by whole body intracardiac perfusion fixation under gravity. First they were deeply anaesthetized with an

overdose of penthotal followed by 0.9% normal saline solution followed with 10% formol calcium fixative, for 25 minutes. Fixation was monitored by the decolourization of the eyeball and tongue. The skull was opened and the whole brain removed enmass and trimmed to the region of the superior colliculus using the stereotaxic coordinate method (Paxinos et al, 1998), then further fixed in 10% formol calcium fixative for 18 hours. The tissue specimens were then processed routinely for histological procedures and sectioning was obtained at 6µ thickness following which respective slide sections were stained for nissls substances using cresyl violet as previously described by Venero et al,2000. Qualitative observations was done on every 10th section from each animal. Using brightfield compound Nikon microscope, YS100 (attached with Nikon camera), the slides were examined and photographed under 100X and 400X objective. Using Image-Pro Express software, count of neurons with prominent nucleolus within a measured rectangular area was performed in the selected regions. The absolute neuronal density per unit area of section for each section obtained was estimated as described by Abercrombie,1946.

***Statistical analysis**:*

The data were analyzed using the computerized statistical package 'SPSS Version 11'. Mean and standard error of mean (SEM) values for each experiment group was determined. The means were compared by analysis of variance at a level of significance of 95% and 99%. Independent samples t-test was performed on each count to determine if there is any statistically significant difference in absolute neuronal count between the control and treatment groups.

RESULTS

***Body weights**:*

Treatment group T1,T2 &T3 showed insignificant increased body weight (**P >0.05**)

of mean±sem (202.4±28.3,207±9.6 & 213± 3.24)g respectively compared to the control group of mean±sem (217.4±7.3g) as seen in **Table I.**

TABLE I
(Mean ± SEM)g of Body weight at the end of administration

Groups	N	Mean ± SEM (g)	D.O.F	2-Prob
T1	10	202.4 ± 28.3*	11.2	0.05
T2	10	207.9 ± 9.6*		
T3	10	213.0 ± 3.24		
C	10	217.4 ± 7.3		

□ ***(P>0.05) Insignificance difference when compared with control using t-test***

Neuronal density

Treatment groups T1 and T2 showed significantly reduced neuronal densities (P<0.05) of Mean±sem (368.3 ± 6.97 and 403.0 ± 10.22)/Sq.cm in T1&T2 **(Table II)** compared to the control section with Mean±sem (719.2 ± 31.7)/Sq.cm. Neuronal density in Treatment in group T3 was also reduced with Mean±sem (601.2 ± 61.3)/Sq.cm. although this is statistically insignificant (P>0.05) compared to the control group. However the percentage reduction in the neuronal densities for T1, T2 and T3 are 48%, 37% and 16% respectively **(Table III).**

TABLE II
Mean ± SEM of Neuronal density per sq. cm of section

GROUPS	Neuronal density per sq. cm
T1	368.3 ± 6.97ϕ
T2	403.0 ± 10.22ϕ
T3	601.2 ± 61.3
C	719.2 ± 31.7

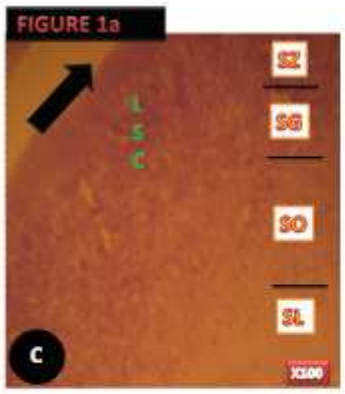
ϕ (P < 0.05) Significance difference when compared with control using t-test ᵠ (P > 0.05) Insignificance difference when compared with control using t-test

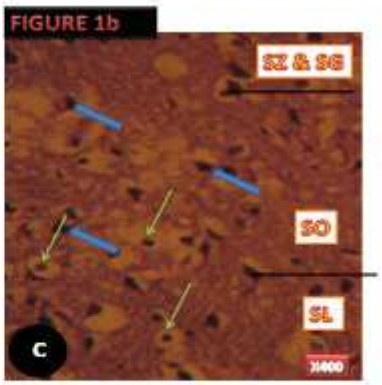
TABLE III
Percentage neuronal loss

GROUPS	% Neuronal loss
T1	48
T2	37
T3	16

Histological findings

Control sections (**Figures 1a and 1b**) showed normal lamina pattern of the superior colliculus with neurons of various shapes ranging from round to oval to pyramidal and intact glial cells well populated especially in the stratum griesieum and stratum optimum.

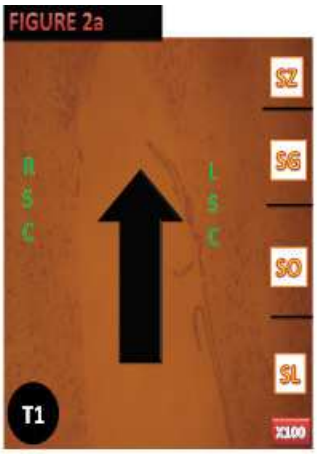




FIGURES Ia & 1b

Photomicrographs of superior colliculus SC(control sections C) of left side (LSC)showing Aqueduct of sylvius (black arrow),distinct layers of SZ(stratum zonale), SG(stratum griseum),SO(stratum optimum) and SL(stratum lemnisci). Note the normal pyramidal neurons(blue arrows) and the evenly distributed glial cells(green arrows) *Nissl stain* x100 & x400 respectively

Treatment group (T1) sections(**Figures 2a and 2b**) revealed clear stratum zonale (**SZ**) and stratum griseum (**SG**) with distorted pyramidal neurons with broken axons mostly in the stratum optimum(**SO**) and stratum lemnisci (**SL**) with sparsely populated neuronal and the glial cells .

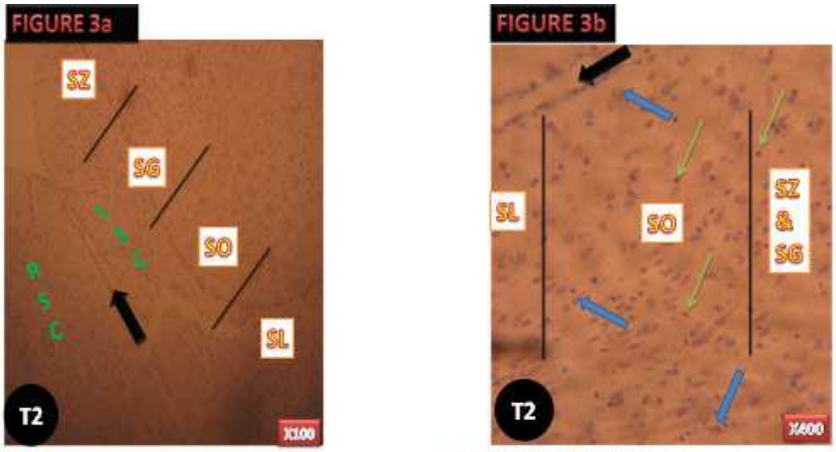


FIGURES 2a & 2b

Photomicrographs of superior colliculus SC(Treatment section T1) of both sides showing Aqueduct of sylvius (black arrow),distinct layers of **SZ,SG,SO** and **SL**. Note the distorted pyramidal neurons(blue arrows) and the scanty glial cells(green arrows) *Nissl stain x100* & x400 respectively

The histological picture in Treatment group (T2) sections (**Figures 3a and 3b**) revealed a seemingly clear stratum zonale and stratum griseum with large mostly pyramidal shaped and sparcely populated

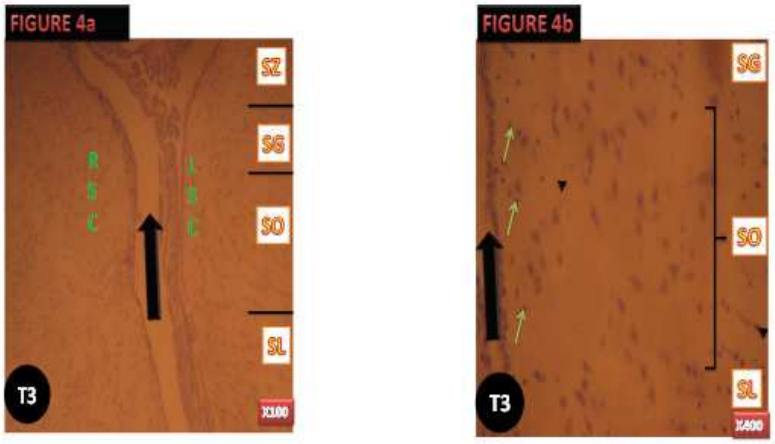
neurons in stratum optimum of which have their axons intact but with distorted cell bodies, the glial cells are also very scanty compared to control sections.



FIGURES 3a & 3b

Photomicrograph of superior colliculus SC(Treatment section T2) of both Right and left (RSC&LSC) showing Aqueduct of sylvius (black arrow),distinct layers of **SZ,SG,SO** and **SL**. Note the few distorted pyramidal neurons(blue arrows) and the scanty glial cells(green arrows) *Nissl stain x100* & x400 respectively

Histological finding on the treatment group T3 (**Figures 4a and 4b**) showed normal neuronal layered pattern of superior colliculus with evenly populated neurons in the stratum zonale and stratum griesieum, most of the neurons in the stratum optimum are clearly visible with centrally located nucleus most of which have lost their axons. No vasculation was noticed although some of the neuronal cell bodies appeared pyknotic but on overall very little neuronal distortion was noticed when compared to the control group. The glial cells most of which are neuroglial are evenly distributed.



FIGURES 4a & 4b

Photomicrograph of superior colliculus SC(Treatment section T3) of both sides showing Aqueduct of sylvius (black arrow),distinct layers of **SZ,SG,SO** and **SL.** Note the less distorted(mostly normal) pyramidal neurons (blue arrows) and the well distributed glial cells(green arrows) *Nissl stain x100* & x400 respectively

DISCUSSION

In the present study aqueous extract of azadirachta indica leaves was administered via oral intubation. The insignificant decrease in the body weights of the treated animals measured as compared to the control implied that azadirachta indica extract consumed have no negative effect on somatic growth. Histological observations noted here revealed distorted pyramidal neurons with broken axons mostly in the stratum optimum (SO) and stratum lemnisci (SL) with sparsely populated neuronal and the glial cells as shown in Figures2B and 3B. Neuronal degeneration has been reported to result in cell death which is of two types namely apoptotic and necrotic. The process of cellular necrosis involves disruption of the membranes structural and functional integrity. Cellular necrosis is not induced by stimuli intrinsic to the cells as in programmed cell death (PCD), but by an abrupt environmental perturbation and departure from the normal physiological conditions(Eweka et al,2009).In cellular necrosis, the rate of progression depends on the severity of the environmental insults. The greater the severity of the insults the more rapid the progression of neuronal injury (Eweka et al,2009). However Treatment groups T1 and T2 showed significantly reduced neuronal densities ($P<0.05$) compared to the control while an insignificant reduced neuronal density was recorded in group T3 when compared to the control is due to effects of chronic intake of neem leaves extract and this finding points to a possibility of dose dependent relationship as the percentage neuronal loss the treatment groups as shown by T1, T2 and T3 were 48%, 37% and 16% respectively (Table III). Lack of cell generation may be a key mechanism of neurodegeneration (Nixon, 2006). Indeed, in many neurodegenerative diseases, the lack of ongoing cell generation by stem cells has been hypothesized to contribute to tissue loss (Armstrong and Barker,2001). New neurons from neural stem cells are constitutively produced in at least two regions of the normal, adult brain. This study had used computer Image-Pro Express software and the sections were stained with nissl's stain which revealed the pyramidal neurons characteristically which were counted with the aid of a digital image software on a computer. Glia ought not to be regarded as 'glue' in the nervous system as the name implies; rather, they are more of a partner to neurons (Andrew,2009).The scanty distribution of the glia cells as noticed in this work will drastically affect their roles of surrounding and holding neurons in place as well as supplying nutrients and oxygen to neurons. Hence the neuronal loss with scanty distribution of the glial cells following chronic consumption of azadirachta indica leaves affects majorly the stratum optimum and stratum lemnisci of the superior colliculus and it can thus be concluded here that chronic intake of neem leaves extract may underline disturbances in orientation and maintenance of saccade

REFERENCES

1. Abercrombie, M. (1946).Estimation of nuclear population from microtome sections. Anatomical Records;94: 239-247
2. Andrews J king (2004) .The superior colliculus Current Biology Volume: 14, Issue: 9, Publisher: Elsevier,Pages: R335-8.
3. Armstrong, R.J. and R.A. Barker (2001). Neurodegeneration: A failure of neuroregeneration? Lancet. 358:1174-6
4. Eweka, A.O. and J.O. Adjene (2008). Histological studies of the effects of oral administration of artesunate on the superior

colliculus of adult wistar rats. Internet J. Trop. Med.,4(2): 1-9.
5. Fujiwara T, Sugishita EY, Takeda Y, Shimizu M, Nomura T, Tromita Y (1984). Further studies on the structures of polysachharides from the bark of Melia azadirachta. Chem. Pharmacol. Bull.,32: 1385–1391.
6. Grantyn A, Berthoz A (1985). Burst activity of identified tecto-reticulo-spinal neurons in the alert cat. *Exp Brain Res* ,57: 417–421
7. Isa T, Sasaki S. (2002) Brainstem control of head movements during orienting; organization of the premotor circuits. *Prog Neurobiol*,66: 205–241 *www.ncbi.nlm.nih.gov/pubmed/11960679*
8. Kausik BI, Ranajet KB (2002). Biological activities and medicinal properties of neem plant. Current Science. 82(11): 1336-1345.
9. Kaushik, N. *et al* (2007).,Regional and habitat variability in azadirachtin content of Indian neem (*Azadirachta indica* A. Jusieu). *Curr. Sci*.,92, 1400–1406.
10. Nayak Aarati,Nayak Ganganath N,G Soumya B,Bath K ishore, Kudalkar mithun (2011). Evaluation of antibacterial and anticandidial efficacy of aqueous and alcoholic extracts of neem(Azadirachta indica). An invitro study.International journal of research in ayurveda and pharmacy.2(1):230-235
11. Nixon, K .(2006). Alcohol and adult neurogenesis: Roles in neurodegeneration and recovery in chronic alcoholism. Hippocampus. 16:287-95
12. Paxinos G and Waston C.(2008). The rats brain in stereotaxic coordinates.Academic press. 4th Ed. San Diego CA
13. Sparks, DL (1999). "Conceptual issues related to the role of the superior colliculus in the control of gaze". Curr Op Neurobiol. 6 (6): 698–707.
14. Yanpallewar SU, Sen S, Tapas S, Kumar M, Raju SS, Acharya SB (2003). Effect of Azadirachta indica on paracetamol-induced hepatic damage in albino rats. Phytomedicine .10(5): 391- 396.
15. Venero, J.L., M.L. Vizuete, M. Revuelta, C. Vargas, J. Cano and A. Machado (2000).Upregulation of BDNF mRNA and trkB mRNA in the nigrostriatal system and in the lesion site following unilateral transaction of the medial forebrain bundle. Exp. Neurol.,161:38–48.