



Reversibility, Body Weight and Histo-architectural Alterations in the Anterior Pituitary Gland of Aqueous *Azadirachta indica* Extract Fed Wistar Rats

P. R. C. Esegbue^{1*}, A. J. Uyovwieseewa², G. T. Olowe² and O. A. Udi³

¹Department of Public and Community Health, Novena University, Ogume, Delta State, Nigeria.

²Department of Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria.

³Department of Anatomy, Achievers University, Owo, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJARR/2019/v6i430157

Editor(s):

(1) Dr. Nadia Sabry El-Sayed El-Gohary, Associate Professor, Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Egypt.

Reviewers:

(1) Jombo Henry Effiong, University of Uyo, Nigeria.

(2) Verisa Chowjarean, Rangsit University, Thailand.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/51307>

Original Research Article

Received 10 July 2019
Accepted 13 September 2019
Published 23 October 2019

ABSTRACT

Several studies have reported the importance of neem plant (*Azadirachta indica*) with results showing it to be both medicinal and pharmacological in the property. However, little or no available record(s) relates its effect on body weights and the anterior pituitary gland. Consequently, the current study investigated the effect of aqueous extract *Azadirachta indica* on the anterior pituitary gland using Wistar rats as an experimental model. Twelve (12) healthy male Wistar rats of between 130 – 150 g were grouped as follows; Group I (normal control) received normal rat feed *ad libitum*, experimental groups II and III received normal feed + 200 mg/kg and normal feed + 300 mg/kg of aqueous leave extract of *Azadirachta indica* respectively. For each group, Body weights were checked weekly for a total of three (3) weeks. At the end of the experiments, the animals were sacrificed, and histology of the pituitary glands was assessed. Result in a statistically insignificant increase ($p < 0.05$) in body weights of control compared with experimental rats, with mild observational elevation in pituitary histo-architecture for experimental compared to the control group. Thus, reversal weights are very supportive factors of Wistar rats and the histological effect

of *Azadirachta indica* on the anterior pituitary gland in Wistar rats. The study also observed axonic fibres (using H and E x 10) with neural tissues in 200 mg/kg extract-treated rats, implicating an increase in cellularity, mild vascular congestion and tissue separation on Wistar rats.

Keywords: *Azadirachta indica*; neem leaf; pituitary gland.

1. INTRODUCTION

In the subcontinent of India, one of the very few known trees is the Neem tree (*Azadirachta indica*) [1,2]. Taxonomically, the Neem tree is classified under the *Meliceae* family, growing rapidly in the tropics and semi-tropical climates of the world. The tree reportedly survives in extremely dry and arid circumstances [1,2] and is incredible to declare in the 21st century by the United Nations [1]. In India, *Azadirachta indica* is variously known as 'Divine Tree', 'Life giving tree', 'Nature's Drugstore', 'Village Pharmacy' and 'Panacea for all diseases'. It is one of the major components in Ayurvedic medicine, which has been practised in India for many centuries.

In Nigeria, Neem tree extract is also called 'Dogonyaro', and is most consistently recommended in ancient medical texts for gut upsets or related conditions like diarrhoea and dysentery, skin ulcers and malaria [3,4].

Available studies suggest parts of the Neem plant such as; leaves, bark, flower, fruit, seed and roots may be advantageous in the management of health issues. For instance, its leaves are reportedly employed as a drug for the treatment of diabetes mellitus, eczema and fever. Its barks have also been shown to be useful in making toothbrushes, while its roots can heal diseases, and are applied in the management of Arachnophylactic shock [5]. Neem seeds have a high concentration of oil, and their oil is widely applicable in industries for the production of insecticides, lubricant and drugs, which are useful in the treatment of a variety of diseases, including tuberculosis [6].

In India, scientific investigations into neem tree are hugely encouraged as part of her program to revitalise India's tradition, plus improve commercial interests on neem [7]. Currently, it has been speculated that little or no other plant (or tree) across the globe has been extensively researched upon, and/or used in all possible capacities than *Azadirachta indica*. In Africa, neem leaf extracts have proved efficacious at

various medicinal preparations [8,9]. *Azadirachta indica* has been of great benefit to human health due to its biochemical, pharmacological, and medicinal properties.

Pioneering works on the possible commercial use of Neem oil and cake had been done by the Indian Institute of Science in Bangalore as early as the 1920s [10]. In the last two decades, research on neem has been intensified and many of the trees agricultural and medical properties were rediscovered. Today, Neem plays a major role in the rural industry of India and projects for the commercial use of Neem have been successfully introduced in other countries [11]. The green pinnate leaves of neem have a very bitter taste and garlic-like smell.

There have been several clinical studies showing that Neem has significant effects on several bacterial strains [12]. Among some of the more prominent strains studied were staphylococcus aureus, streptococcus pyogenes, cornebacterium, *E. coli*, and *Salmonella typhosa*. These bacteria can cause meningitis, cystitis, sore throats, typhoid, blood poisoning, and food poisoning. Neem's ability to exert significant effects over the above mentioned bacterial strains indicates its ability to resolve the aforementioned conditions. Though *Azadirachta indica* is also reportedly used by many individuals for antimalaria therapy or management, this study is conducted as a sub-part of a wider independent study that seeks to assess the possible physiological effects of the plant on the brain.

1.1 Aim of the Study

Several types of research have been carried out on neem plant (*Azadirachta indica*) with results showing it to be both medicinal and pharmacological in usage. However, little or no documentation currently relates the anterior pituitary effect of the leaf of the plant. Using Wistar rat as an experimental model, this work was consequently designed to examine the effect of aqueous extract of neem leaf on the anterior pituitary gland.

2. MATERIALS AND METHODS

2.1 Resources and Sources

Over 20 g of fresh neem leaf was purchased in the month of July 2013 from local markets in Abraka main market, Delta State, Nigeria. With assistance from expert taxonomists, obtained samples were then identified to be neem plant (*Azadirachta indica*) at the Department of Animal and Environmental Biology, Delta State University, Abraka.

2.2 Study Design

Twelve adult rats Wistar breed weighing between 130 g-150 g were procured from the Animal House of the Ambrose Alli University, Ekpoma, Edo State. The rats were housed in wire mesh cages under standard laboratory conditions with standard feed with free access to water *ad libitum* in the same Animal house. They were allowed to acclimatize for four (4) weeks before the start of the experiment, following which they were divided into three (3) groups of four (4) rats each;

Group 1: Normal experimental control (positive control) was fed with normal feed and water daily for 28 days.

Group 2: Known as the experimental group was treated with 200 mg/kg of neem leaf (*Azadirachta indica*) extract and fed with normal feed and water for 28 days.

Group 3: Known as the reversal group was fed with normal feed and water daily for 28 days.

2.3 Sample Preparation and Extraction

The sample of the neem leaves (*Azadirachta indica*) was obtained in the month of July 2013, from Abraka main market, Delta State. The neem leaves were removed from the stem and washed thoroughly with clean tap water, cut into slices with a knife for easier extraction and allowed to air-dry for some days under room temperature. After proper drying, 200 g of the dried leaves sample was weighed out and pounded.

Extraction of the neem leaves was carried out using 20 g of the ground leaves the sample in soxhlet extractor with distilled water in the Pharmacognosy Department of Emma-maria Biomedic Laboratories and Consultancy, Abraka. The recycling of the solvent was allowed to be repeated for complete extraction. The slurry

extract was then poured into the evaporating dish to evaporate the solvent in the extract over the water bath at the temperature of 70°C – 75°C and a yield of 22.5 g of crude extract were obtained.

2.4 Determination of Body Weights

Using the electronic weighing balance, rat's body weights were determined before and after treatment with test substance every week and the day of sacrificing. This was recorded in grams. The experimental animals were also often observed for signs of abnormalities throughout the study.

2.5 Sample Collection from Animal

After an overnight fast by the rats, the rats were sacrificed by placing each rat in a desiccator containing cotton wool soaked with chloroform and covered until they become anaesthetized. Each rat was placed on the dorsal surface and a laparotomy was carried out to expose the internal organs, the anterior pituitary gland was collect put in a universal container containing 10% formol saline for fixation of the tissue for histological determination.

2.6 Preparation of Tissue for Microscopic Examination

The process of preparation of the anterior pituitary gland for histological examinations is separated into the following stages:

2.7 Fixation

After the Wistar rats in each group were sacrificed by the use of a chloroform desiccators, the anterior pituitary gland from all groups was carefully removed. It was then fixed in a 10% formal saline for 48 hours.

2.8 Tissue Processing

This is a preparatory treatment that entails impregnation of the specimen with an embedding medium to provide support and suitable consistency for microtomy. The stages include dehydration, impregnation and embedding.

Dehydration entails removal of water from the tissue by passing it through graded solutions of alcohol from 70% to 100%. Tissues were passed from low concentration (70%) to high concentration (100%). This was to allow for complete dehydration and prevent turbulence. In

a clearing, anterior pituitary gland was treated with xylene a substance which is both miscible with alcohol and molten paraffin wax. The anterior pituitary gland was passed in three different changes of paraffin wax. Embedding is also called casting. This was done by filling a mould with molten paraffin wax, orienting the liver tissue in the mold to ensure it is cut in the right plane and finally cooling the mass to promote solidification.

2.9 Sectioning and Mounting

The anterior pituitary gland was processed using the paraffin wax method with an automatic tissue processor by the following schedule;

- i. 70% alcohol for 2 hours
- ii. 90% alcohol for 2 hours
- iii. 95% alcohol for 2 hours
- iv. 2 changes of absolute alcohol for 2 hours each
- v. 2 changes of xylene for 2 hours
- vi. 2 changes of paraffin wax for 2 hours each
- vii. 2 samples were embedded in paraffin wax at 70 degree centigrade and cut with a rotary microtone 4 μ

2.10 Tissue Staining

The staining technique employed in this study was the haematoxylin and Eosin staining techniques. It is comprised of the following stages;

- i. De waxing and dehydration
- ii. Staining in Erlich's haematoxylin for 15minutes
- iii. Rinsed in water for 15 minutes
- iv. Blue in tap water for 2 minutes
- v. Counter stained with 1% Eosin for 1 minute
- vi. Dehydrated, cleared and mounted

2.11 Photomicrography

Stained tissue images were captured using digital microscopic eyepiece "SCOPEK" DCM

500, 5.0 mega pixels connected to USB 2.0 computer.

2.12 Ethical Issues

Experimental protocols were executed in strict compliance with the commendations and guides for the care and use of laboratory animals. The study adhered to the code of conduct stipulated by the Institute for Laboratory Animal Research.

2.13 Statistical Analysis

The results were expressed as mean \pm SEM (Standard error of the mean) and statistical significance of the treatment effect was analysed using the student's t-test statistics (LSD t-test), one way analysis of variance (ANOVA), followed by post HOC LSD test for multiple comparisons, using software social science (SPSS) version 20 windows software and significance at p values < 0.05 while p value > 0.05 were considered to be statistically non-significant.

3. RESULTS

Table 1. Shows the effect of *Azadirachta indica* leaves extract on the bodyweight of Wistar rat were expressed in mean \pm SEM. From Table 4, bodyweight of normal rats (control group 1) was significantly (P<0.05) increasing when initial (156.80 \pm 3.53) where compared with final weight (177.40 \pm 3.69), similar result were observed in reversal (group 3) initial (177.90 \pm 4.14) where compared with final weight (174.93 \pm 5.70) at (P<0.05), but not significance.

3.1 Photomicrograph of Anterior-Pituitary Gland

Fig.1. Showing the histological effect of *Azadirachta indica* leaves extract on the anteriorpituitary gland of Wistar rats.

4. DISCUSSION

The result from the current study is presented in the Tables in Chapter four. From the Tables, it is

Table 1. Change in body weight of control Wistar rats

Control group body weight (G)			
	Initial	Final	Weight change
1	163.00	178.30	15.3
2	157.30	179.30	22
3	160.10	184.80	24.7
4	146.80	167.20	20.4
N=4	156.8 \pm 3.53	177.4 \pm 3.69	20.6 \pm 1.98

Values are expressed as mean \pm Standard error of mean (S.E.M), n=4

Table 2. Changes in body weight due to reversibility effect of *Azadirachta indica* leaves extract in Wistar rats

	Experimental group body weight (G)		
	Initial	Final	Weight change
1	182.80	165.00	17.8
2	185.30	183.50	1.8
3	166.70	165.20	-1.5
4	176.80	186.00	9.2
N=4	177.90±4.14	174.925±5.70	6.83±4.29

Values are expressed as mean ± Standard error of mean (S.E.M), n=4

Table 3. Effect of *Azadirachta indica* leaves extract on body weight of Wistar rats

Groups	Initial weight (g)	Final weight (g)	Percentage change (%) body weight
Control	156.80±3.53	177.40±3.69*	11.61
Reversal	177.90±4.14	174.93±5.70	-1.70

Values are expressed as mean ± Standard error of mean (S.E.M), n=5. *p <0.05: Percentage change between initial and final weight determine

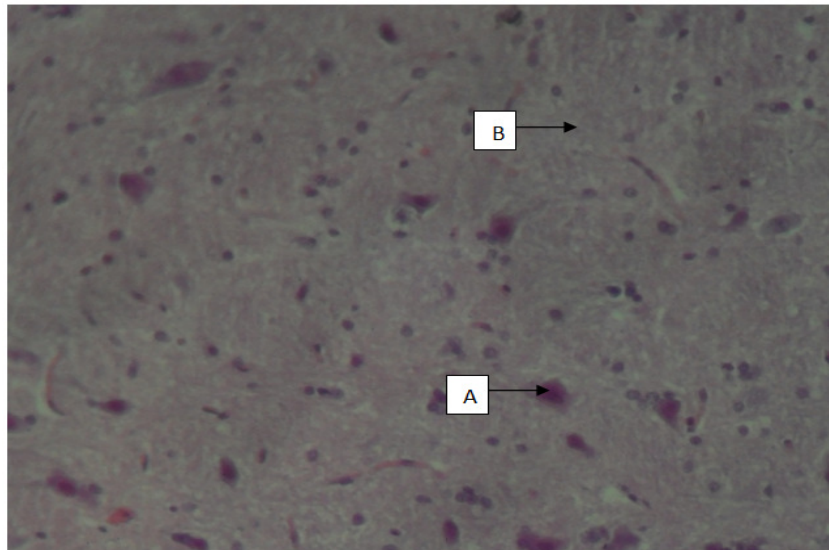


Figure 1. Control: Rat Neural tissue showing glial cells A and axon fibres B (H&E x 10)

seen that the aqueous extract of *Azadirachta indica* mildly affects the bodyweight which is not significant when compared to control group which is in agreement with Obikaon et al. (2012) [13] that mildly reduce body weight. And the rat Neural tissue treated with 200 mg/kg aqueous extract of *Azadirachta indica* showing increase cellularity, mild vascular congestion and tissue separation (H&E x 10) in Fig. 2, The interpretation of this is that the continuous intake of the aqueous extract of *Azadirachta indica* leaves to improve the conduction of impulses along the axons of the central nervous system. Another review also agree and state that aqueous leaf extract of *Azadirachta indica* increase sodium and potassium ions are vital

electrolytes in the transfer of information along with the central nervous system [14-16]. Lack of these electrolytes indicates serious deleterious effect to the physiological homeostasis of higher organisms, especially man. On another note, sodium ions play an important role in the transport of glucose across the plasma membrane [17]. Glucose is the cellular-preferred metabolite for the generation of energy through the glycolytic pathway. This metabolite would not cross the cellular membrane barrier unaided. It is, therefore, transported to where it is metabolized in exchange for sodium ions. This shows that lack of this vital electrolyte (example sodium ion) could 'ordinarily' lead to the starvation of cells [18].

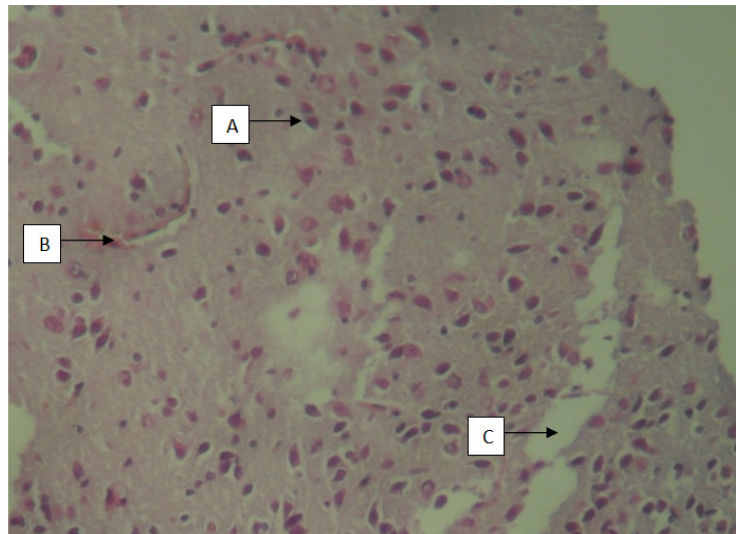


Figure 2. Rat Neural tissue treated with 200mg/kg Neem leaf extract showing increase cellularity A, mild vascular congestion B and tissue separation C (H&E x 10)

The result of this study also revealed mild pathological effects in the anterior-pituitary gland of the rats treated with 200 mg/kg of *A. indica* leaf extract. This is in line with Upadhyay *et al* (1990) who reported normal neural tissue morphologies, and functions with the seed oil extract of *A. indica* [19,20]. This also corroborates the work of Prakasha *et al.* (1988) who earlier reported normal histo-architecture of the brain cell of rats treated with neem oil extract [21].

5. CONCLUSION

In conclusion, 200 mg/kg of aqueous extract of the leaf of *A. indica* does not have any obvious effect on the histo-morphologies of the anterior-pituitary gland, but showed mild changes in the bodyweight of Wistar rat, implying that the effect of the extract may have been at a level other than these organs of study.

6. RECOMMENDATIONS

It is therefore recommended that future research should study the mechanism of actions of the extract to their effect on the health of the brain. The extract should also be purified and graded for further clinical studies.

ETHICAL APPROVAL

Animal Ethics committee approval was obtained before carrying out the study.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Amabe O. Akpantah, Moses B. Ekong, Kelechi C. Uruakpa, Mfon Akpaso, Mokutima A. Eluwa, Theresa B. Ekanem. Gonadal histo-morphologies and serum hormonal milieu in female rats treated with *azadirachta indica* leaf extract. Iranian Journal of Reproductive Medicine. 2010; 8(4):185-190.
2. Anyaehie USB. Changes in some physiological parameters of HIV/AIDS patients on antiretroviral and neem (*Azadirachta indica*) extract in Enugu, Nigeria. Ph.D. Thesis, Abia State University, Uturu; 2009.
3. Bhattacharyya KG, Sharma A. *Azadirachta indica* leaf powder as an effective biosorbent for dyes: A case study with aqueous congo red solutions. Journal of Environmental Management. 2004;71(3): 217-22.
4. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). Curr. Sci. 2002;82(11):1336–1345.
5. David PH, Hommel M, Miller LH. Udeinya IJ, Oligino LD. Parasite sequestration in plasmodium falciparum malaria: Spleen and antibody modulation of cytoadherence

- of infected erythrocytes. Proc NaH. Acad. Sci. 1983;80(16):5075-5079.
6. Ekanem OJ. Has *Azadirachta indica* (Dogonyaro) any antimalarial activity? Niger Med. J. 2001;8:8-11. Gandhi, M. (1988). Acute toxicity study of the oil from *Azadirachta indica* seed (neem oil). Journal of Ethnopharmacology. 23(1):39-51.
 7. Ganguli S. Neem: A therapeutic for all seasons. Current Science. 2002;82(11): 1304.
 8. Obikaonu HO, Okoli IC, Opara MN, Okoro VMO, Ogbuwu IP, Etuk EB, Udedibie ABI. Haematological and serum biochemical indices of starter broilers fed leaf meal of neem (*Azadirachta indica*). Journal of Agricultural Technology. 2012; 8(1):71-79.
 9. Johnson S, Morgan ED, Peiris CN. Development of the major triterpenoids and oil in the fruit and seeds of neem (*Azadirachta indica*). Journal of Annals Botany. 1996;78:383-388.
 10. Masoumeh MM. Sterility effects of neem (*Azadirachta indica*) extract on male rat. Journal of Reproduction and Infertility. 2002;3(2):10.
 11. Mbah AU, Udeinya IJ, Shu EN, Chijioko CP, Nubila T, Udeinya F, Muobuikie A, Mmuobieri A, Obioma MS. Fractionated neem leaf extract is safe and increases CD4⁺ cell levels in HIV/AIDS patients. AM. J. Ther. 2007;14(4):369-374.
 12. Bhuiyan MM, Nishimura M, Matsumura S, Shimono T. Antibacterial effects of the crude *Azadirachta indica* neem bark extract on streptococcus sobrinus. Pediatr Dent J. 1997;7(1):61-64.
 13. Mongkholkhajornsilp D, Douglas S, Douglas PL, Elkamel A, Teppaitoon W, Pongamphai S. Supercritical CO₂ extraction of nimbin from neem seeds-A modeling study. Journal of Food Engineering. 2004;71: 331-340.
 14. Natarajan V. Effect of *Azadirachta indica* (Neem) on the growth pattern of dermatophytes. Indian Journal of Medical Microbiology. 2003;21(2):98-101.
 15. Nwafor SV, Akah PA, Okoli CO, Onyirhioha KC, Nwosu CS. Interaction between chloroquine sulphate and aqueous extract of *Azadirachta indica* a. juss (Meliaceae) in rabbits. Acta Pharm. 2003;53:305-311.
 16. Ofusori DA. Regenerative potential of aqueous extract of neem *Azadirachta indica* on the stomach and ileum following ethanol- induced mucosa lesion in adult wistar rats. The Internet Journal of Nutrition and Wellness. 2008;5(2).
 17. Prakasha AO, Tewaria RK, Mathur R. Non-hormonal post - coital contraceptive action of neem oil in rats. J Ethnopharmacol. 1988;23:53-59.
 18. Ragasa CY, Nacpil ZD, Natividad GM, Tada M, Coll JC, Rideout JA. Tetranortriterpenoids from *Azadirachta indica*. Journal of Phytochemistry. 1997; 46:555-558.
 19. Raveendra MP. Evaluation of antiplaque activity of *Azadirachta indica* leaf extract gel- a 6week clinical study. Journal of Ethnopharmacology. 2004;90(1):99-103.
 20. Schmutterer H, Ascher KRS, Isman MB. The neem tree: *Azadirachta indica* a. juss and other meliaceous plants. Weinheim. 1995;1-3.
 21. Stix G. Village pharmacy: The neem tree yields products from pesticides to soap. Sci. Am. 1992;266:132.

© 2019 Esegbue et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/51307>