SOME EFFECTS OF AQUEOUS LEAF EXTRACT OF *Terminalia glaucescens* ON THE MICRO-ARCHITECTURE OF THE HISTOLOGY OF CEREBRUM OF WISTAR RATS

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ABSTRACT

Medicinal herbs are in great demand in the developed countries as well as developing countries because of their wide biological and medicinal activities, higher safety margins and lesser costs. Terminalia glaucescens is a deciduous, multipurpose perennial tree that grows across Africa especially South-West Nigeria. The leaves extract of this plant is used in medicinal preparations for the treatment of AIDS, amenorrhea, scrofulous infections, syphilis, sores and nervous diseases. The effects of this plant on various body organs has not been fully documented, hence, the aim of this research which is to determine some effects of aqueous extract of *Terminalia glaucescens* on the microarchitecture of the cerebrum of Wistar Rats. Fifteen rats weighing between 150-200 g were divided into three groups of 5 animals each. Animals in group 1 served

as control and they received distilled water, animals in group 2 received 200 mg/kg of extract each and animals in group 3 received 300 mg/kg of extract each. At the end of four weeks, animals were sacrificed by cervical dislocation, the cranium was assessed and the cerebrum was removed. The cerebrum was fixed using 10% formolsaline for histological stain (H&E stain).
In this study, it was shown that *Terminalia glaucescens* has a significant effect on the body of animals in group 2 at week 4 as opposed to animals in group 3, but it is not dose dependent. The study also shows that the aqueous extract of *Terminalia glaucescens* has no toxic effect on the histology of the cerebrum but enhances its development has shown by the numerous pyramidal neurons.

INTRODUCTION

Medicinal herbs are in great demand in the developed countries as well as developing countries as they were having the awareness of primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs (Adeeyo *et al.,* 2012). Most developing countries, including Nigeria are endowed with vast resources of medicinal and aromatic plants and these plants have been used over the millennia for human welfare (Rukangira, 2001). Throughout history, plants have been the principal source of drug used in preventing and curing of diseases and in the production of some drugs currently used in modern medicine. The use of higher plants and their extracts to treat infection is an age-old practice in African medicine and it is an effective practice in many third world countries (Olorundare *et al.,* 1992). Medicinal plants represent a rich source from which antimicrobial agents may be obtained.

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The active components of many drugs found in plants are secondary metabolites (Sofowora, 1982). Traditional medicine practices in Nigeria have continued to provide remedies for various diseases (Adelakun *et al.,* 1999) and most rural dweller depends on it for their health care needs (Ekpa and Ebana, 1992). This is because of the variety of herbal preparations that can be made from plants to treat different kinds of ailment including microbial infections (Akinyemi *et al.,* 2000).

MATERIALS AND METHODOLOGY

Animal Model

Fifteen (15) youngwistar rats of average weight between 20g-30g were obtained and housed in standard cage under standard condition. They were fed with pellet standard rat diet and water and were kept in the animal house.

Plant Material

The Moringa Oleifera leaf was obtained in Ogbomosho, Oyo State, Nigeria. The leaf was identified and authenticated at the botany departmentof Pure and Applied Biology of Ladoke Akintola University of Technology. The plant material was dried for Four weeks, pulverized and grinded to achieve a fine powder form. The powdery form was taken to the department of Food science and Engineering of Ladoke Akintola University of Technology where extraction was carried out. The Moringa powder was soaked in distilled water for 24hrs, after which it was evaporated at temperature of 105˚C. The extract is then mixed with distilled water and used for administration.

Experimental Design

After the rats were procured, they were weighed and acclimatizedfor two weeks;Group 1 comprises of five(5) wistar rats and serves as control group and they were fed with standard diet and distilled water.Group 2 comprises of five (5) wistar rats and serves as the group given 100mg/kg/bw of aqueous leaf extract moringa and also fed with pellet and water. Group 3 comprises of five(5) wistar rats and serves as the group given 200mg/kg/bw of aqueous leaf extract moringa and also fed with pellet and water.

Administration

The route of administration was orally by using the oral cannula and the administration of moringa Oleifera took six (6) weeks.

Histological Technique

Fixation: The harvested lungs were fixed immediately in 10% formol saline. The purpose of fixation is to preserve tissues permanently in as life-like a state as possible (Weiss et al., 2010). The lungs were fully immersed into the 10% formol saline as soon as possible after the removal of the lungs to prevent autolysis.

Dehydration: After fixation, the lungs were removed and subjected to dehydration. The tissues were dehydrated in ascending grades of alcohol as follows:
70% of alcohol – 1 hr, 80% of alcohol – 1 hr, 90% of alcohol – 1 hr, 95% of alcohol – 1 hr, 99% of alcohol – 1 hr, Absolute alcohol -1 hr.

Clearing: The next step is called "clearing" and involves removal of alcohol. The tissues were cleared using xylene1&2 (for 15 minutes each) as one of clearing agents to clear away the alcohol.

Infiltration/Impregnation: The tissues were impregnated with molten paraffin wax. This was to make the tissue hard and water resistant (Wells, 1988). The above process was performed twice for 1 hour each using the automated tissue processing machine, although they can be perform manually.

Embedding :This is a process by which liquid paraffin wax is use to solidify the tissues in the embedding moulds by the use of plastic embedding cassette with a pair of warm forceps so as to give a desired result. The tissue blocks were removed and air dried until the wax becomes solidified.

Sectioning: The tissue was cut into various thicknesses from 5 to 10 microns with the use of rotary microtome. But before sectioning, the tissue blocks was properly placed into a block holder of the microtome and trimmed to expose the surface of the tissue (Presnell *et al*, 1997).

Floating: The cut section was gently lowered using fine forceps into the surface of warm water in a water bath at a regulated temperature. When the section is fully expanded and flattened, clean, grease – free slide was dipped obliquely into the water bath to pick up the section. The

slides was labeled with a diamond pencil and transferred into an incubator for the slide to completely dry.

Staining: This was done using hematoxylin and eosin. After taking the section to water in order to dehydrate them, section were stained regressively in hematoxylin for 5 minute. Excess hematoxylin was removed using 1% acid alcohol consequently removing its blue color which was retrieved by blueing in slow running water. Eosin was used to counterstain the sections.

Staining Procedure for Hematoxylin and Eosin (H&E): The tissue section was de-waxed by immersing the slides in xylene for 2-3 minutes. After being de-waxed, the slides were immersed into a descending grades of alcohol (2 changes of absolute alcohol, 90% and 70% alcohol) and finally in a distilled water. The slides were stained in Hematoxylin, The slides were washed in running tap water for 5 – 10 minutes, The slides were then stained in Eosin for 1 – 2 minutes, The slides were dehydrated in ascending grades of alcohol, After removing from absolute alcohol, the slides were cleared in xylene.

Mounting: The slides were mounted in Dibutylpthalate xylene (DPX) and covered slipped.

Statistical Analysis

Data were analyzed using Excel 2007. Data were expressed as mean +/- standard error of the mean (Mean +/- SEM). Mean values were compared using one way analysis of variance (ANOVA). P value less than 0.05 ($P<0.05$) were taken to be statistically significant. All graph were drawn with Excel 2007.

HISTOLOGICAL OBSERVATION

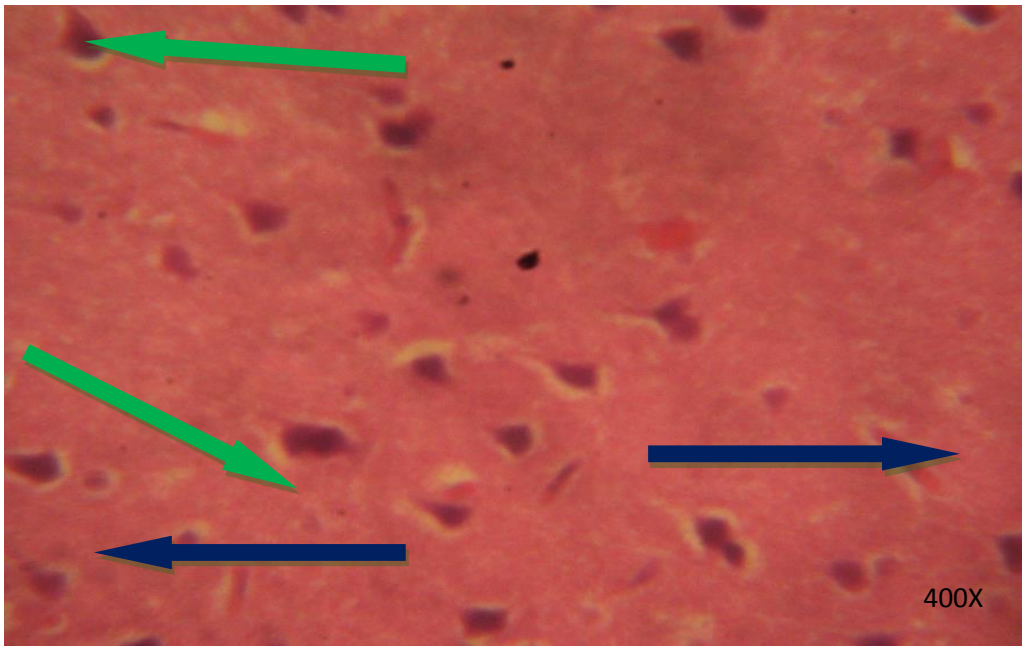

PLATE A: Light Photomicrograph of control slide of prefrontal cortex: the control notes normal evenly distributed pyramidal neurons (green arrow), normal perivascular space and glial cells (blue arrow).

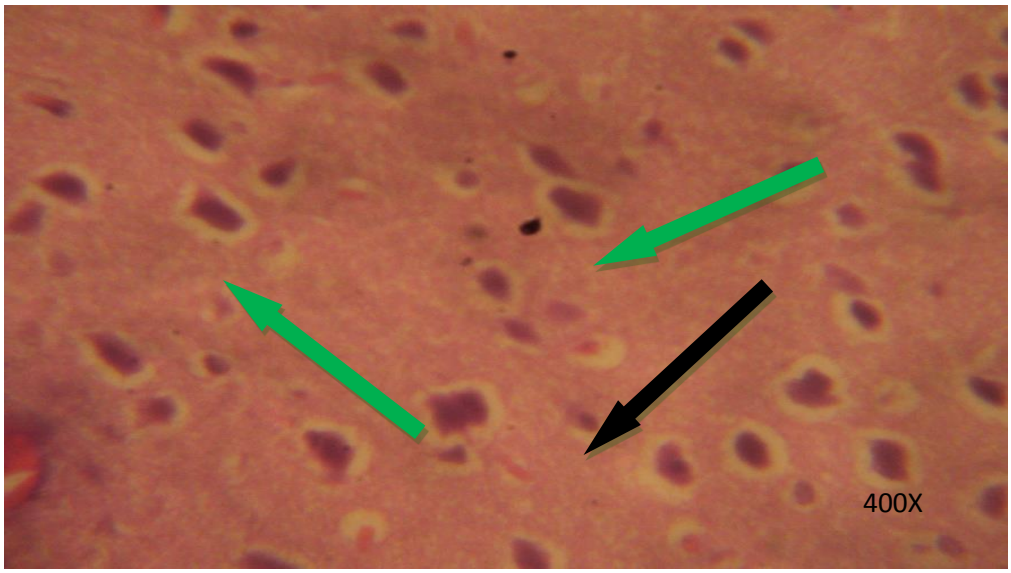

PLATE B: Light Photomicrograph sections of prefrontal cortex of the cerebrum (H&E) X400 of the animals administered with 200/mg/kg of Terminalia glaucescens showed numerous pyramidal neurons(green arrow), and glial cells appear normal(black arrow) and increased perivascular space.

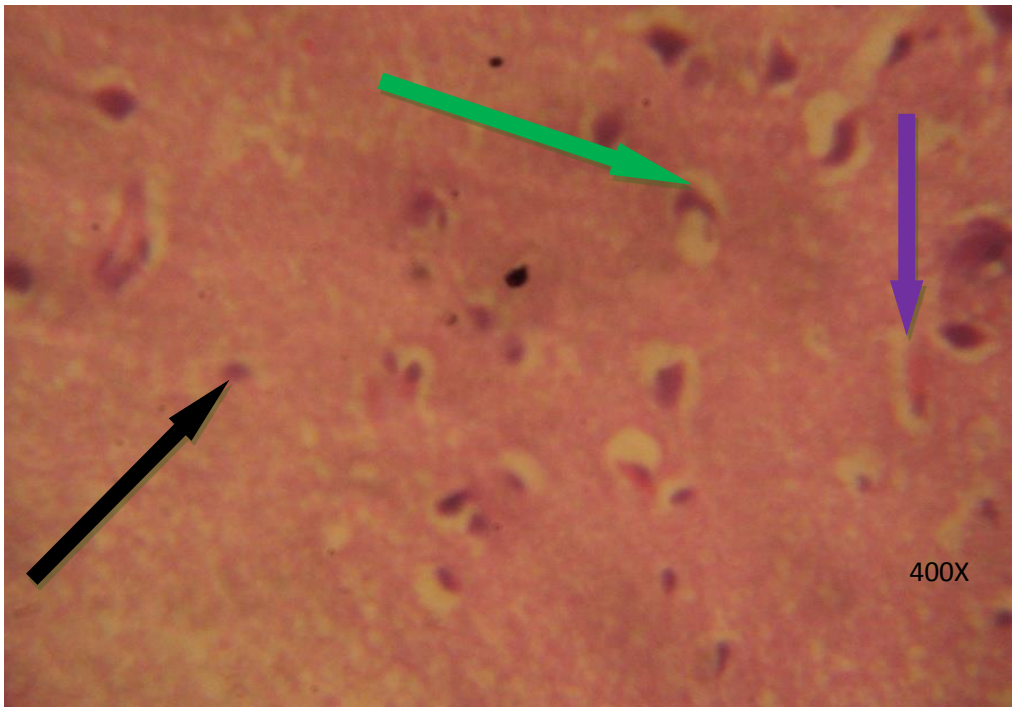

PLATE C: Light Photomicrograph of prefrontal cortex of the cerebrum (H&E) X400 of animals administered with 300mg/kg of Terminalia glaucescens showed scanty pyramidal neurons(green arrow), pyknotic pyramidal neurons(purple arrow) and glial cells appear normal(black arrow) and increased perivascular space.

Table 1: Mean and Standard error of Mean of Animals

	Week 1	Week 2	Week 3	Week 4
Group 1 (control)	170.0 ± 30.00	166.0 ± 26.51	162.0 ± 21.94	175.0 ± 20.92
Group 2	123.0 ± 6.442 0.1641	124.0 ± 6.595 0.1635	125.0 ± 11.18 0.1714	117.0 ± 5.148 0.0274*
Group 3	135.0 ± 6.124 0.2861	136.0 ± 5.788 0.3020	131.0 ± 4.848 0.2051	140.0 ± 4.743 0.1413

Pvalue – *significant difference

The result here shows there was a significant difference in the mean weight of the animals in group 2 compared to the control group at the fourth week of administration.

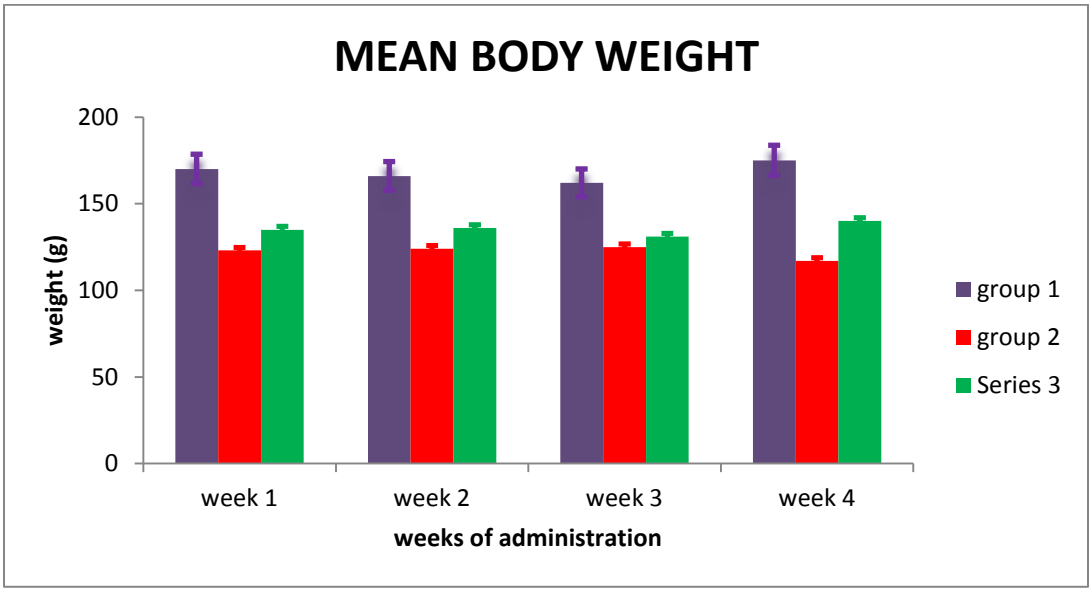

Fig III – Chart showing the mean and standard error of mean weight of animals.

DISCUSSION

The use of herbs to treat disease is almost universal among non-industrialized societies, and it is often more affordable than purchasing more expensive pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become interestingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds (Tapsel *et al*., 2006).

Terminalia glauscecens is a deciduous, multipurpose perennial tree that grows across Africa especially South-West Nigeria. The leaves extract of this plant is used in medicinal preparations for the treatment of AIDS, amenorrhea, scrofulous infections, syphilis, sores and nervous diseases (Koudou *et al*., 1995).

This project report shows some of the effects of aqueous leaf extract of *Terminalia glaucescens* on the micro-architecture of the histology of cerebrum of wistar rats. In this study, the varying doses of aqueous leaf extract of *Terminalia glaucescens* has a significant effect on the body of animals in group 2 at week 4 as opposed to animals in group 3, but it is not dose dependent. Animals administered with low dose of *Terminalia glaucescens* showed numerous pyramidal neurons, and glial cells appears normal and increased perivascular space, animals administered with high dose of *Terminalia glaucescens* showed scanty pyramidal neurons, pyknotic pyramidal neurons and glial cells appear normal and increased perivascular space. This shows that high dose of *Terminalia glaucescens* causes reduction in pyramidal cells.

High dose of *Terminalia glaucescens* appears to have no significant effect on the glial but causes increase of perivascular space while it decreases the number of pyramidal cells. Low dose of *Terminalia glaucescens* increases both the number of pyramidal cells and the perivascular space while the glial cell remains normal. It has no significant effect on the animals in group 3 but has a significant effect in animals in group 2 at the fourth week of administration.

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