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Research Article

# Histomorphological responses to aqueous crude leaf extract of *Alafia barteri* on prefrontal cortex, heart, kidney, liver and testis of adult male Sprague-Dawley rats

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## ABSTRACT

Phytonutrients present in *Alafia barteri* leaves include antioxidants which serves to protect cells and tissues against detrimental effects of reactive oxygen species and other free radicals. This research work was targeted at investigating the activities of oral administration of aqueous leaf extract of *Alafia barteri* on the histology of the prefrontal cortex, heart, kidney, liver and testis of adult Sprague Dawley rats. Twelve (n=12) adult male Sprague Dawley rats weighing between 170-200 g (4-6 weeks old) were used for this study; they were divided into 2 groups of six rats each. The control group A received 2 ml/kg normal saline and treated group B received 500 mg/kg body weight aqueous extract of *Alafia barteri* for twenty eight days. The gross anatomical parameters of the selected organs and their histology were assessed. The gross anatomical and histological observation of the prefrontal cortex, heart, kidney, liver and testis revealed no visible distortion in *Alafia barteri* extract treated group when compared with control. Aqueous leaf extract of *Alafia barteri* thus has no deleterious effects on the histological profile of the prefrontal cortex, heart, kidney, liver and testis of the rats.

**Keywords:** *Alafia barteri*; Frontal cortex; Heart; Kidney; Liver; Testis.

## 1. INTRODUCTION

The importance of herbs in the treatment of diseases is almost universal among non-industrialized societies, and is often more assessable and affordable compared to modern pharmaceutical drugs. The World Health Organization (WHO) estimated that 80 percent of the populations of some Asian and African countries presently use herbal medicine to treat various ailments. Biological compounds present in *Alafia barteri* leaves include antioxidants which serves to protect cells and tissues against detrimental effects of reactive oxygen species and other free radicals. Protective agents from plant origin with anti-peroxidative and antioxidant properties play an important role in protecting the liver against toxicity [1, 2].

*Alafia barteri* has been used in traditional medicine to treat various diseases in Nigeria and other African countries since time immemorial. *Alafia barteri* Oliv, Apocynaceae, is a climbing

shrub distributed widely in the tropics. It is valued for its efficacy in the traditional medicine system in Nigeria and other African countries, as an anti-inflammatory and fever remedy. The infusion of the leaves and twining stem are used for the treatment of inflammation and fever [3, 4]. The decoction of root and leaves of the plant is also taken internally or applied externally to treat rheumatic pain, toothache and eye infection [5]. The extracts of the leaves were found to have antibacterial and antifungal activities [6, 7]. The aqueous leaf extract was reported to display potent antiplasmodial activity [8], antinociceptive and anti-inflammatory activities [9].

In South-Western Nigeria (specifically in Lagos), *Alafia barteri* has been used for the treatment of malaria [10]. Apocynaceae is quite a large family with about 200 genera and 2000 species known, including genus like *Alafia*, *Catharanthus*, *Alstonia*, etc. [11]. Plants in the Apocynaceae are poisonous, rich in alkaloids, glycerides and flavonoids obtained from the leaves, seeds, stems, roots and latex and are known source of anti-malarial activities [12, 13]. *Alafia barteri* Oliver (Hook F. Icon) is a tropical rainforest plant, native to the West and Central Africa, stretching from Guinea Bissau to Cameroon, Congo and Nigeria [11]. *Alafia barteri* is called agbari-etu by the natives of South-Western Nigeria (Lagos), meaning instant fever remedy. Leaf infusion and root decoctions from *Alafia barteri* are used in Nigeria and other African countries as a remedy for malaria [14]. In Nigerian traditional medicine, the stem and root decoctions of *Alafia barteri* are used for treating rheumatic pains, toothache, eye infection and sickle-cell anaemia [10, 12]. Polyphenols, flavonoids and alkaloids have been reported for wide varieties of pharmacological activities, including antiplasmodial activity [15-17]. High levels of polyphenols and flavonoids reported in the roots and leaves fractions of *Alafia barteri* could be responsible for its antiplasmodial activity [8].

To this end, we employed histological methods to evaluate the safety use of *Alafia barteri* leaf extracts on selected vital body organs. The rationale is that histological observations would provide a more assertive and reliable results of the effects produced as a result of the interactions between phytochemicals and body tissues than *in vitro* tests and analysis of the highly dynamic biochemical activities as contained in extracted tissue fluids. In addition, the use of Histological methods of assessment of the effects of *Alafia barteri* leaf extract on body tissues is important since literatures are comparatively scarce on such methods of investigation of the plant's extracts' effects.

Present study therefore focused on the effects of *Alafia barteri* leaf extract on histo-architecture of prefrontal cortex, heart, kidney, liver and testis of male Sprague-Dawley rats.

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant material

The leaves of *Alafia barteri* were collected in December 2017 at Ipale forest, Irawo, Atisbo Local Government, Oyo State, Nigeria. The plant sample was authenticated by professor Ogunkunle of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Voucher specimen deposited in the same unit for reference purpose.

### 2.2. Preparation of the plant extract

The leaves were thoroughly washed in sterile water and were air dried to a constant weight in the laboratory. The air-dried leaves were weighed using Gallenkamp (FA2406B, England) electronic weighing balance and were milled with automatic electrical Blender (model FS-323, China) to powdered form. The powdered plant sample (500 g) was extracted with 96% ethanol for 24h, at room temperature with constant stirring. This process was repeated twice for complete extraction. The extract was filtered through cheese cloth and then through Whatman #1 filter paper, the filtrate was concentrated using a rotary evaporator (Rotavapor® R-210) at 42-47°C.

### 2.3. Animals and treatment

Male wistar rats 8 weeks old, weighing 170 ± 200 g were obtained from the animal facility of Department of Anatomy, Ladoke Akintola Uni-

versity of Technology, Ogbomoso, Nigeria. The animals were kept in polypropylene cages under room temperature (25°C), with 12 h light and 12 h dark cycle and were allowed to acclimatize for two weeks. The animals were fed with grower's mash (Farm support services Ltd, Ogbomoso, Nigeria) at a recommended dose of 100 g/kg as advised by the International Centre of Diarrheal Disease Research, Bangladesh (ICDDR, B) daily. They had access to water *ad libitum*. Twelve male wistar rats (n=12) were used for the investigation. They were divided in two groups of Control (A) which received only 2 ml/kg normal saline and Treated (B). A daily dosage of 500mg/kg body weight of *Alafia barteri* extract was administered orally to the treated Group B for 28 days. Twelve hours after the administration of the last *Alafia barteri* dose, the rats were at the time of sacrifice first weighed, blood samples were collected through ocular artery and centrifuged at 1,500 g/min at 4°C for 10 min to obtain serum then animals were sacrificed under high ether anaesthesia. All experimental protocols followed the guidelines for Care and Use of Laboratory Animals in Biomedical Research of the National Institutes of Health of the United States [18] and Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria Ethical Committee guide line.

### 2.4. Histology preparation of the organs

The organs were harvested and fixed in formaldehyde for 24 h after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% and absolute alcohol and xylene for different durations before they were transferred into two changes of molten paraffin wax for 1 hour each in an oven at 65°C for infiltration. They were subsequently embedded and serial sections cut using rotary microtome at 5 microns. The tissues were picked up with albumenized slides and allowed to dry on hot plate for 2 min. The slides were dewaxed with xylene and passed through absolute alcohol (2 changes); 70% alcohol, 50% alcohol and then to water for 5 min. The slides were then stained with haematoxylin and eosin. The slides were mounted in DPX. Photomicrographs of the tissues were taken.

## 3. RESULTS

### 3.1. Morphological observations

There was increased in body weight in both the experimental animals administered with *Alafia barteri* extract and control group throughout the duration of the experiment. In addition, there were no morphological alterations in the appearance of the prefrontal cortex of the brain, heart, kidney, liver and testis of the animals in the treatment groups compared to those in the control groups twenty four hours after the organs were harvested. The prefrontal cortex, heart, kidney, liver and testis (with all their component parts) of the animals in both the treatment and control groups appeared morphologically normal.

### 3.2. Histology observations of prefrontal cortex, heart, kidney, liver and testis tissues

The neurohistological assessment of the frontal cortices of the rats in the extract treated group displayed normal histological profile, degenerative changes such as cytoarchitectural distortions, vacuolations and evidence of necrotic bodies were absent in the frontal cortices of the extract treated rats. The sections obtained in the control group shows numerous intact pyramidal cells with their nuclei (Fig. 1).

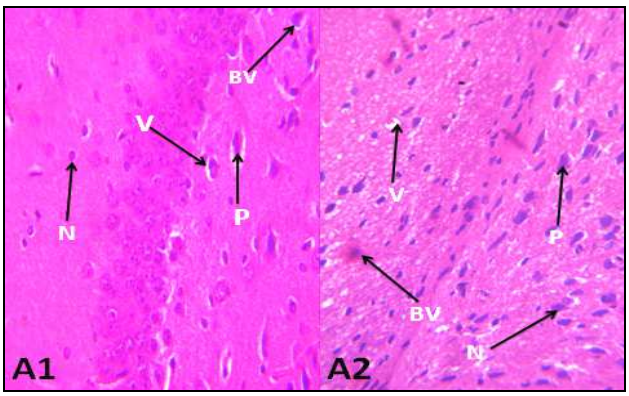


**Figure 1.** Images of the prefrontal cortex of the animals in control A1 and tread A2 groups. (H&E, x 400). V = vacuolations, P = pyramidal cells, Bv = Blood vessels, N = neurons. Numerous intact pyramidal cells with their nuclei.

The cardiac histology in both control and extract treated group revealed a normal appearance showing normal and centrally arranged nucleus, connective tissue also appeared normal the cardiac muscle fibers are well arranged (Fig. 2).

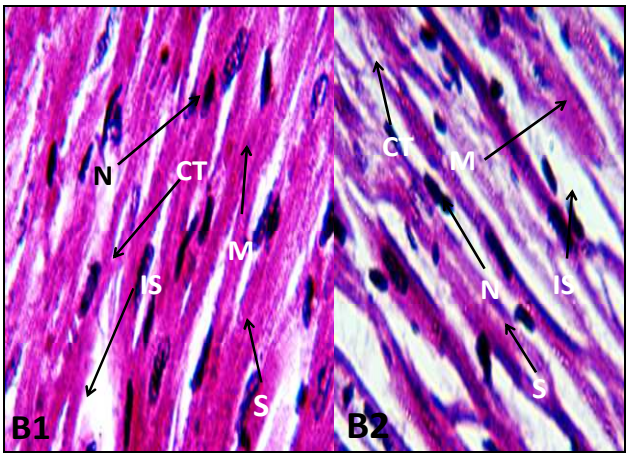


**Figure 2.** Histological demonstration of the photo-micrograph of section of the heart in control B1 and treated group B2 at light microscope level using H&E staining techniques (x 400) showing, normal Nucleus (N), normal space striation (S), normal connective tissue (CT), normal muscular fibre (MF) were well arranged.

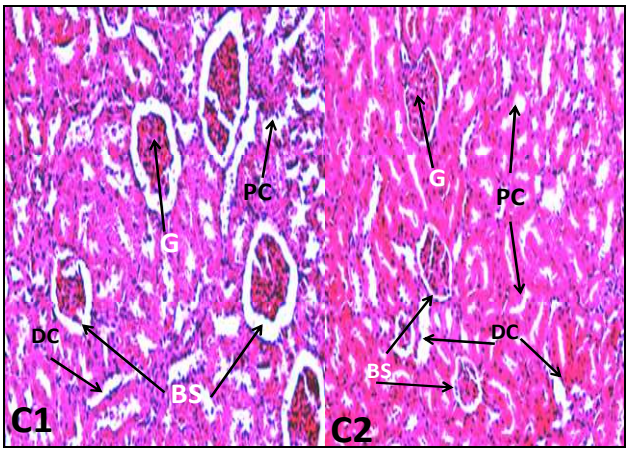


**Figure 3.** Histological demonstration of the photo-micrograph of section of the Kidney in control C1 and treated group C 2 at light microscope level using H&E staining techniques (x 400) showing normal and preserved histological outline with normal glomerulus (G), Bowmen's space (BS), proximal convoluted tubules (PC) and distal convoluted tubules (DC).

The histological outline of the kidney of the rats in the treated and control group appeared normal and preserved (Fig. 3). The sections of the liver of the rats in both the extract treated and the control groups also displayed well preserved histological profile with evidence of normal hepatic cytoarchitecture with visible terminal hepatic lobules consisting of terminal hepatic venules, hepatocyies with intervening sinusoidal spaces radially accentuated (Fig. 4).

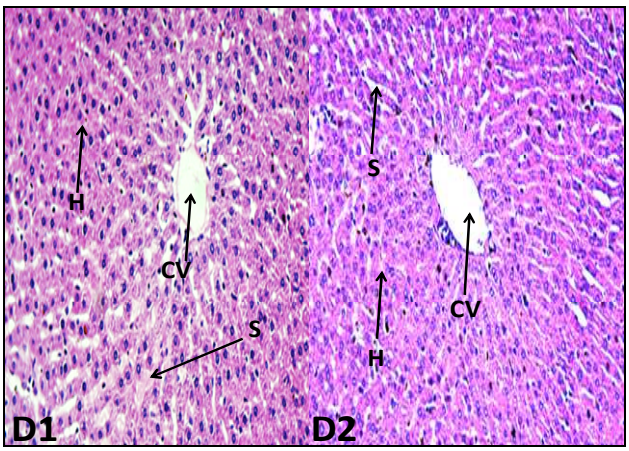


**Figure 4.** Histological demonstration of the photo-micrograph of section of the Liver in control D1 and treated group D2 at light microscope level using H&E staining techniques (x 400) showing the normal central vein (CV) sinusoids (S) and hepatocytes (Hc).

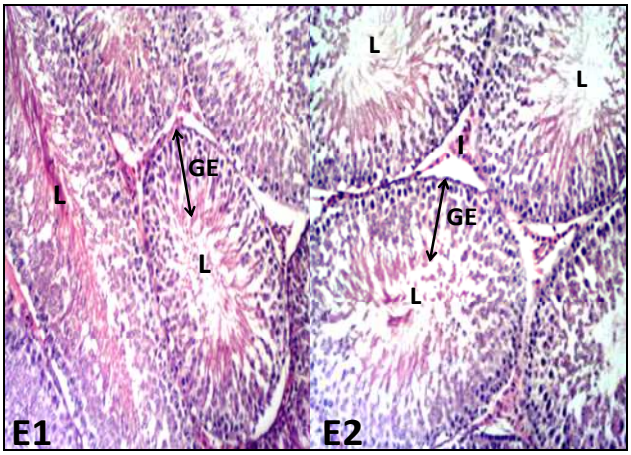


**Figure 5.** Histological demonstration of the photo-micrograph of section of the testes in control E1 and treated group E2 at light microscope level using H&E staining techniques (x 100) showing normal cellularity in germinal epithelium (GE), lumen (L) filled with sperm cells and interstitial cells of Leydig in the interstitium (I).

The histological section of the testes of the rats in both the extract treated and the control groups devoid of histo-pathological abnormalities

and revealed a normal cellular composition in their germinal epithelium with sperm cells in the lumen and a normal interstitium (Fig. 5).

## 4. DISCUSSION

*Alafia barteri*, which is the plant of interest in this study, is used by some African populations for its nutritional and pharmacological properties [19]. In this study, we investigated some of the effects of the aqueous leaf extract of *Alafia barteri* on prefrontal cortex, heart, kidney, liver and testis in order to elucidate some of the possible implications that could occur following its consumption.

Histomorphology observation on the prefrontal cortex following administration of aqueous extracts of *Alafia barteri* revealed normal histoarchitecture with intact cells and their nuclei, this could be ascribed to the presences of bioactive constituents present in the *Alafia barteri* extract such as flavonoid, terpenoids, saponin, tannins, steroid and cardiac glycoside which are antioxidant agent, this concur with the report of Makajuola et al. [2] that plant with antioxidant constituents improved histomorphology of the prefrontal contex. The histomorphology of the heart of the control and *Alafia barteri* extract treated group demonstrates normal morphology which is in consonances as reported by Ajibade et al. [20] that the cardiac histology of the rats treated with physiological saline revealed a normal appearance showing normal and centrally arranged nucleus, connective tissue also appeared normal and cardiac muscle fibers are well arranged. The kidney's functional integrity is to maintain total body homeostasis through its role in the excretion of metabolic wastes and in regulation of intracellular fluid volume, electrolyte composition, and acid-base balance [21]. This therefore implies that any harmful effect on body metabolism could be suggestive of toxic insult to the kidney [22]. The histological observations seen in the sections of the kidney of the experimental rats in the treated groups stained with H&E revealed that oral administration of the aqueous leaf extract of *Alafia barteri* has no deleterious effects on the histological outline of the kidney in this study. Therefore histological appearance of the control and treated group is consistent with normal histology. The histological observations seen in the sections of the liver in the control and *Alafia barteri* extract treated group revealed normal hepatic cytoarchitecture with evident of visible terminal hepatic lobules consisting of terminal hepatic venules, hepatocyies with intervening sinusoidal spaces radially accentuated. Since *Alafia barteri* has antioxidant components it can protect and alter any damage done to the liver by heavy meters or microorganism. This is in line with the report of Ibegbu et al. [23] that the results of histological observations showed normal architecture of the liver with central vein, hepatic cords and sinusoidal spaces in groups of animals treated with physiological saline.

There is no any observable lesion in the histology of the testes in the extract groups when compared with the control. This is in accordance as reported by Cody et al. [24], Harborne and Williams [25] that plants containing flavonoids are effective in prevention of lesion, mainly because of their antioxidant properties. However, in test groups, there was an observed increased in spermatogenic activity towards the lumen of the seminiferous tubule. This increased cellular activity was from the basement membrane up to the lumen of the seminiferous tubules of the testes. This was evidenced by the reduced number of primary spermatogonia cells. This is an indication that they might have differentiated to next level of spermatogenic cells mainly due to the presence of potent antioxidant like flavonoids that scavenge free radicals and increase testosterone formation by the interstitial cells of Leydig [26]. Our observations are therefore concur with the report of Muhammed et al. [27], Ofusori et al. [28] and Adekomi [29]. Cell death occurred pathologically or accidentally is regarded as necrotic and could result from extrinsic implications or disturbances to the cell which may include toxic or traumatic effects [30]. Processes involved in cellular necrosis may lead to cell death include compromise or disruption of the structural and functional potentials of the various membranes in the cell. Necrosis of the cell is not induced by intrinsic stimuli to the cells as observed in programmed cell death, but by an abrupt environmental disturbances and deviation from the normal physiological conditions, factors and functions. The type of cell loss and the particular part of the organ affected determines the symptoms associated with individual disease [31]. This study thus shows that

oral administration leaf extract of *Alafia barteri* has no disruptive and toxic impacts on cellular characteristics of the frontal cortex, heart, kidney, liver and testis of Sprague Dawley rats. To the best of our knowledge, this is the first study reporting the impact of *Alafia barteri* on the histological profile of the selected organs of study in Sprague Dawley rats.

## AUTHORS' CONTRIBUTIONS

SA, OB and OD contributed in collecting plant samples and identification, running the laboratory work and analysis of the data. SA and OB contributed to biological studies and analysis of the data. SA and OD contributed to critical reading of the manuscript. SA designed the study, supervised the laboratory work and wrote manuscript. All authors read and approved the final manuscript.

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## TRANSPARENCY DECLARATION

Authors have declared that no competing interests exist.

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