

RESEARCH PAPER

HISTOLOGICAL EFFECT OF *ALLIUM SATIVUM* ON THE LUNGS OF ADULT WISTER RAT

¹IDEHEN, I.C., ²DIC-IJIEWERE, O.E., ¹AIRHOMWANBOR, K.O., ¹EIDANGBE, A.P., ³NWAOPARA, A.O., ¹UWUIGBE, M., ¹ALAR-ENOMA, O.

¹Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Medical Sciences, Ambrose Alli University, Private Mail Bag 14, Ekpoma-Nigeria. ²Department of Chemical Pathology, Faculty of Clinical Sciences, College of Medical Sciences, Ambrose Alli University, Private Mail Bag 14, Ekpoma-Nigeria. ³Department of Anatomy, College of Medical Sciences, Ambrose Alli University, Private Mail Bag 14, Ekpoma-Nigeria.

Correspondence: ebenexar@gmail.com

Published: 31st January, 2017

Endorsed By: Innovative Science Research Foundation (ISREF) and International Society of Science Researchers (ISSCIR).

Indexed By: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society; and IRMS Informatics India (J-Gate)

ABSTRACT

Allium sativum (Family-Liliaceae), commonly known as 'garlic', is an ancient plant with well known culinary and medicinal properties. Its toxicity potentials however, remain a subject of scientific investigation; especially when consumed in excess. This study therefore, was intended to determine the effects of garlic powder on the histology of the lungs in 30 adult Wister rats. The rats were randomly selected into six groups (A, B, C, D, E and F) and allowed to acclimatize for a period of two weeks; with free access to food and water. They were subsequently subjected to a treatment period of three weeks, during which groups B, C, D, E and F (test groups), received garlic-supplemented basal diets containing 200mg, 400mg, 600mg, 800mg and 1000mg of garlic powder respectively. Group A however (control), received basal diet only. At the end of the treatment period, the lung tissues were harvested and fixed as appropriate for routine tissue processing and microscopy with a binocular light microscope. The results showed no histological alterations in the lung tissue sections; suggesting the pulmonary safety of *Allium sativum* on rats at doses less than or equal to 1000mg.

Keywords: *Allium sativum*, Garlic, Wister rats, toxicity, lungs

INTRODUCTION

Allium sativum (Family-Liliaceae) is a medicinal plant which is widely cultivated in Shan State in Myanmar. It is commonly known as garlic. The bulb has been used for culinary and medicinal purposes in many countries since ancient times; though the ancestry of cultivated *Allium sativum* is not definitively established according to Daniel *et al.*, (2000). It is a treatment of fever, cough, asthma, flatulence, arthritis, skin disease, hypertension, diabetes mellitus (Paul, 1996) and said to possess hypolipidemic, hypocholesterolaemic, antimicrobial, antihypertensive, hypoglycaemic, hepatoprotective, antioxidant and anticoagulant activities (Nagakawa *et al.*, 1995). Oral administration of petroleum ether extract of garlic bulb to rats significantly prevented the rise in serum cholesterol and triglyceride levels caused by arthrogenic diet (Konjuri *et al.*, 2007).

Garlic belongs to the plant genus *Allium*, and is known for its pungency and spiciness. It is thought, in particular, to be linked to various beneficial health effects, from reducing blood pressure and cholesterol to treating cancer. It has also been said to possess antifungal, antibacterial, cardio-protective and antioxidant activities. Several compounds are



involved in garlic's possible anticancer effects. Two main medical ingredients responsible for the health benefits of garlic include allicin and diallyl sulphides (Garlic-central, 2011).

The phytochemicals responsible for the sharp flavour of garlic are produced when the plant's cells are damaged. When a cell is broken by chopping, chewing, or crushing, enzymes stored in cell vacuoles trigger the breakdown of several sulphur-containing compounds stored in the cell fluids. The resultant compounds are responsible for the sharp or hot taste and strong smell of garlic. Some of the compounds are unstable and continue to evolve over time. Among the members of the onion family, garlic has been by far the highest concentrations of initial reaction products, making garlic much more potent than onions, shallots, or leeks (Kodera, 1989). Although many humans enjoy the smell and taste of garlic, these compounds are believed to have evolved as a defensive mechanism, deterring animals like birds, insects, and worms from eating the plant (Yamasaki, 1991).

Although the health benefits of garlic are frequently reported, excessive intake can have harmful effects. In a rat study, allicin, the main pungent ingredient in garlic, was found to be an activator of Transient receptor potential ankyrin 1 (TRPA1). The neurons release neurotransmitters in the spinal cord to generate pain signals and neuropeptides at the site of sensory nerve activation, resulting in vasodilation as well as inflammation (Fleischauer and Arab, 2001). Garlic is known to cause halitosis as well as the pungent 'garlicky' smelly sweat due to allyl methyl sulfide (AMS). AMS is a gas which is absorbed into the blood during the metabolism of garlic; from the blood it travels to the lungs (and from there to the mouth causing bad breath) and skin where it is exuded through skin pores. Washing the skin with soap is only a partial and imperfect solution to the smell. Other side effects include headache, itching, garlic odour on breath and skin, occasional allergic reactions, stomach disorders and diarrhea, decrease in serum protein and calcium levels, association with bronchial asthma, contact dermatitis and complaints of garlic smell (Fleischauer and Arab, 2001). This study therefore, examines the effects of garlic powder on the histology of the lungs in 30 adult Wister rats

MATERIALS AND METHODS

Geographical Description of the study area; The project was carried out in the Department of Medical Laboratory Science, College of Medicine, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Edo State. Ekpoma is a major semi-urban town in Esan West Local Government Area of Edo State. Edo State, South-South, Nigeria, and lies between longitude 06° 04'E and 06° 43'E and latitude 05° 44'N and 07° 34'N. The town has a land mass of 17, 450 sq.km and a population of about 3.1 million people (World Gazetteer, 2007). The residents are either farmers, traders, civil servants or students.

Experimental Animals: Thirty (30) Wister rats supplied from Animal house of College of Medicine, Ambrose Alli University, were used in this study, having a body weight range in between 0.15kg -0.25kg, irrespective of sex. They were housed in labeled cages. The animals were fed basal diet containing pellet feeds and grains and tap water. They were allowed to acclimatize for two weeks.

Substance Preparation: Garlic was procured from the market at Emaudo, Ekpoma, Edo State. After the removal of the outer coverings, the garlic was sliced, sun dried and blended into fine powder using an electric blender. The fine powdered garlic was measured using an Electric Weighing Balance. The measurement was done separately; each packed in a drug envelope from which aliquots were reconstituted with distilled water to obtain semi-solid form for proper consumption.

Research Design: The Wister rats were divided into six groups each containing five rats (n=5) with weight ranging between 0.15kg-0.35kg. The six groups which were tagged groups A, B, C, D, E and F were fed in the following order for three weeks;

1. Group A: Basal diet and water only.
2. Group B: Basal diet, water and 200mg of garlic powder.
3. Group C: Basal diet, water and 400mg of garlic powder.
4. Group D: Basal diet, water and 600mg of garlic powder.
5. Group E: Basal diet, water and 800mg of garlic powder.
6. Group F: Basal diet, water and 1000mg of garlic powder.



These amount of garlic was greater than the known LD₅₀ value of 625.08mg/kg rat intra-peritoneally (Nwanjo and Oze, 2006). No data was found for the LD₅₀ for Garlic in rats orally.

In order to ensure that each animal took the accurate milligrams of garlic powder, the garlic powder was made into aliquot with distilled water and with the aid of a sterile Pasteur pipette, it was carefully administered orally to the Wister rats.

Sample Collection: At the end of the three weeks feeding, the animals were euthanized and their lungs harvested and fixed in different appropriately labeled containers containing 10% formal saline. They were subsequently processed and stained with haematoxylin and eosin for microscopy.

Materials/Equipment's and Reagents Used: Materials/Equipment's and Reagents used for this study include dissecting set, small plastic containers, tissue cassettes, tissue basket, automatic tissue processor (LEICA TP 1020), hot plate, paraffin wax, metallic embedding mould, hot plate, hot air oven, rotary microtome and water bath. The reagents used included 10% Formal saline, Harris Haematoxylin, 1% acid alcohol, Eosin, 10% Formalin, DPX and different grades of alcohol (70%, 80%, 90% and absolute alcohol).

Tissue Processing: The lung tissues were processed with 18 hours automatic tissue processor (LEICA TP 1020) and the procedures in the machine are as follows: Beaker 1-10% formalin for 1hr 30mins, Beaker 2-70% alcohol for 1hr, Beaker 3-80% alcohol for 1hr, Beaker 4-90% alcohol for 1hr, Beaker 5- 95% alcohol for 1hr, Beaker 6- 95% alcohol for 1hr 30mins, Beaker 7- Absolute alcohol I for 2hr, Beaker 8-Absolute alcohol II for 2hr, Beaker 9-Xylene I for 1hr 30mins, Beaker 10- Xylene I for 1hr 30mins, Wax bath I for 2hr, and Wax bath II for 2hr. After the liver tissues were processed, they were embedded in molten paraffin wax with the use of plastic embedding moulds. The embedded tissues were placed inside the refrigerator to effectively solidify. They were trimmed after removing them from the embedding mould to expose the surface of the tissues. The tissues were then placed on an ice block to cool the surface of the tissue so as to allow easy and effective cutting of ribbon of sections from the tissues. The tissue sections were cut at 5µm with the use of rotary microtome. The tissues were placed on clean grease free slides and floated in the water bath (400C) with the use of 20% alcohol. The sections were picked from the water bath, well labeled using a diamond pencil and placed on a hot plate to allow the section to stick properly to the slide so as to avoid lifting during staining. Haematoxylin and Eosin technique was used to stain the tissue sections to demonstrate general tissue structure.

Microscopic Examination: The mounted slides were examined using x10 and x40 objectives of a binocular light microscope. Microscopy was done for all the sections and the results were compared with the control sections before photomicrography.

RESULT

In comparison with the control tissue sections of group A that presented normal histological features of the lung, the microscopic examination of the tissue sections of test group B, C, D, E and F, revealed no visible necrotic changes, portal congestion/hemorrhage or loss of cellular architecture (*See Fig. 1 – 6*).

Table 1: Table showing summary of histological findings

S/N	Histological Effects Examined	Experimental Groups/Doses					
		A(0mg)	B(200mg)	C(400mg)	D(600mg)	E(800mg)	F(1000mg)
1	Portal congestion/Hemorrhage	-	-	-	-	-	-
2	Necrosis	-	-	-	-	-	-
3	Loss of cell architecture	-	-	-	-	-	-

Keys: mg = Milligram; - = Absent



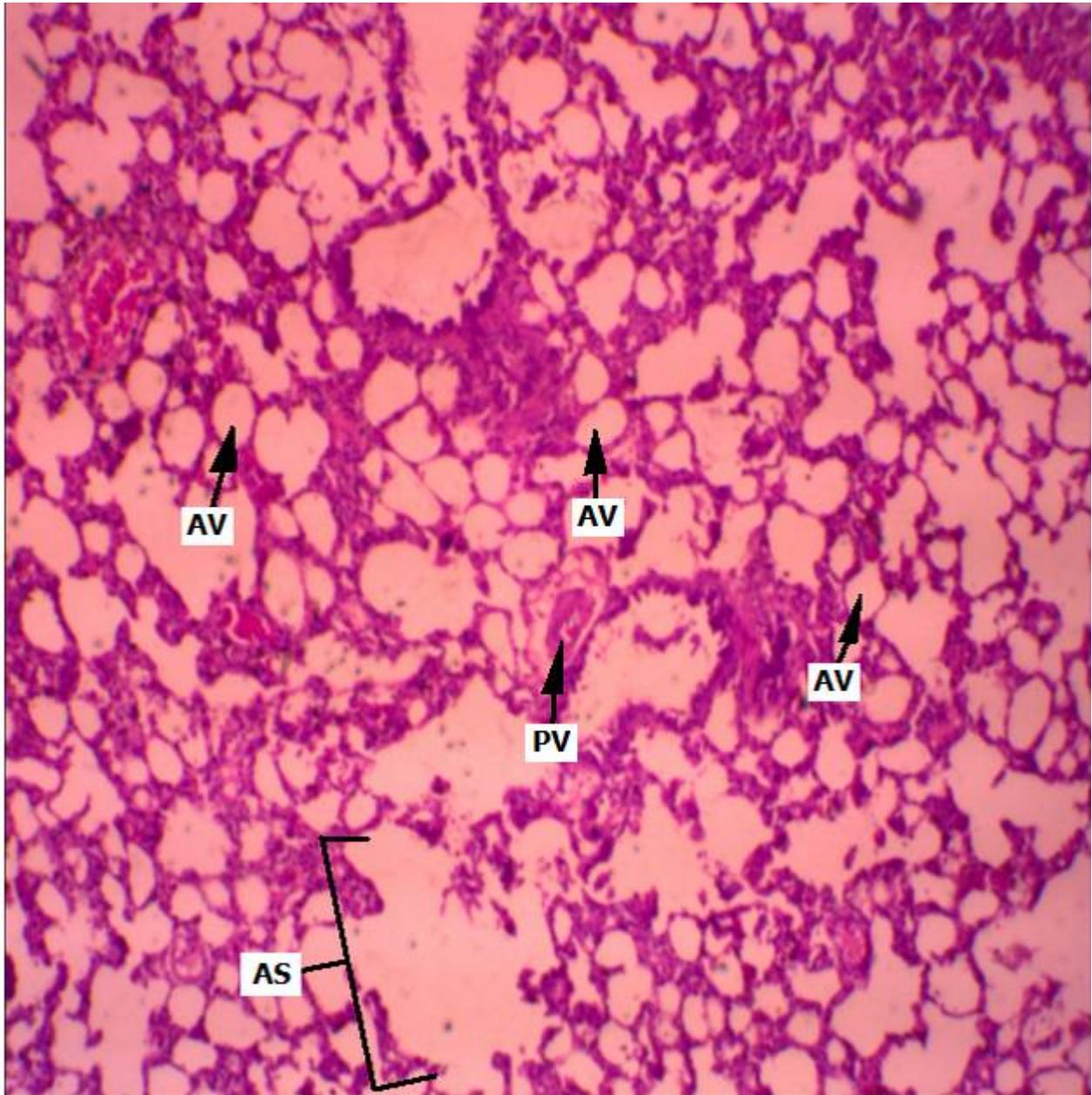


Fig. 1: Photomicrograph of control rat lung tissue section (H&E x100) showing normal lung cytoarchitecture with intact alveoli (AV), alveolar sacs (AS) and pulmonary vessel (PV).

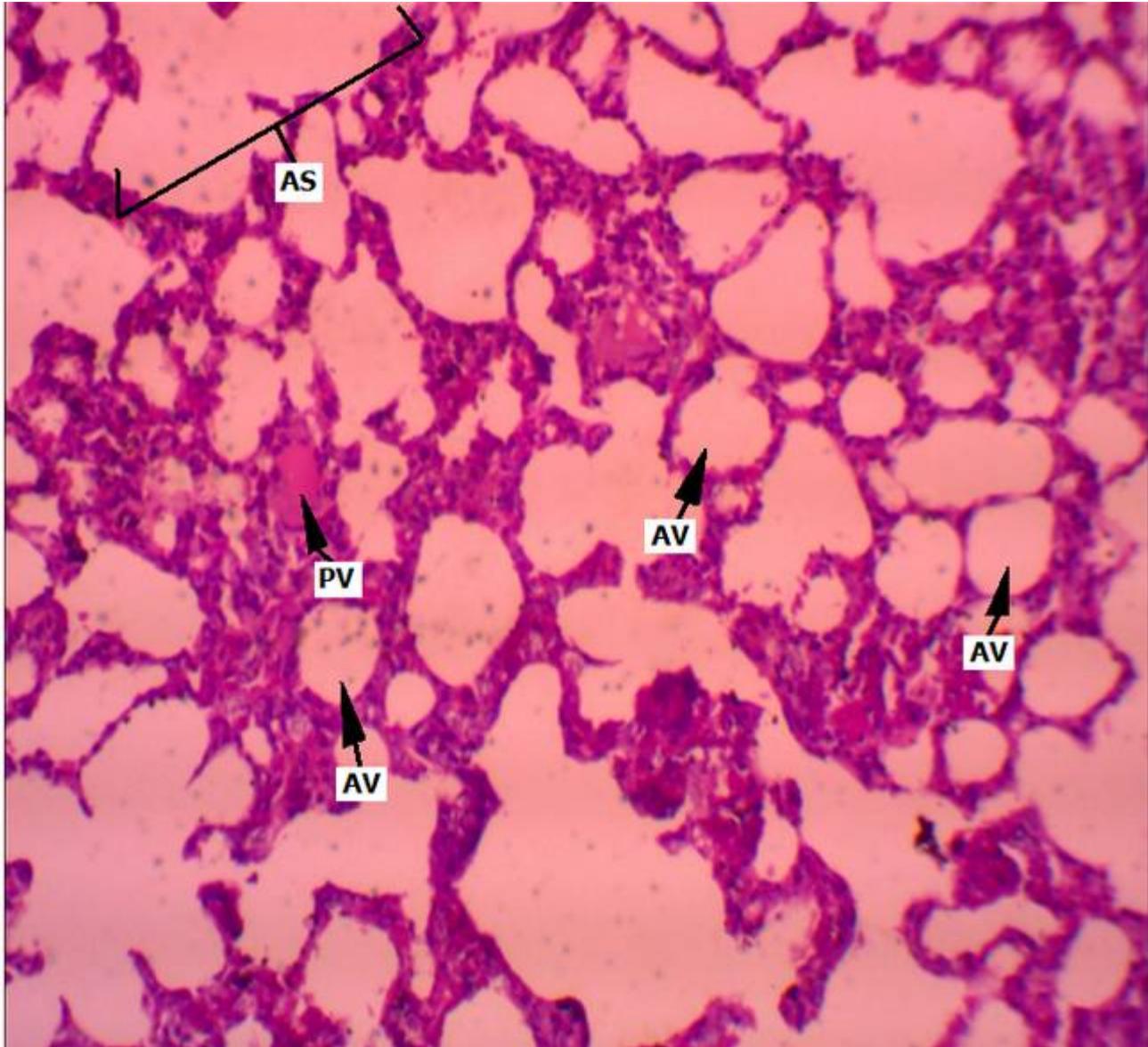


Fig. 2: Photomicrograph of test group B (200mg) rat lung tissue section (H&E x100) showing intact cytoarchitectural features of the lung with visible alveoli (AV), alveolar sacs (AS) and pulmonary vessel (PV).



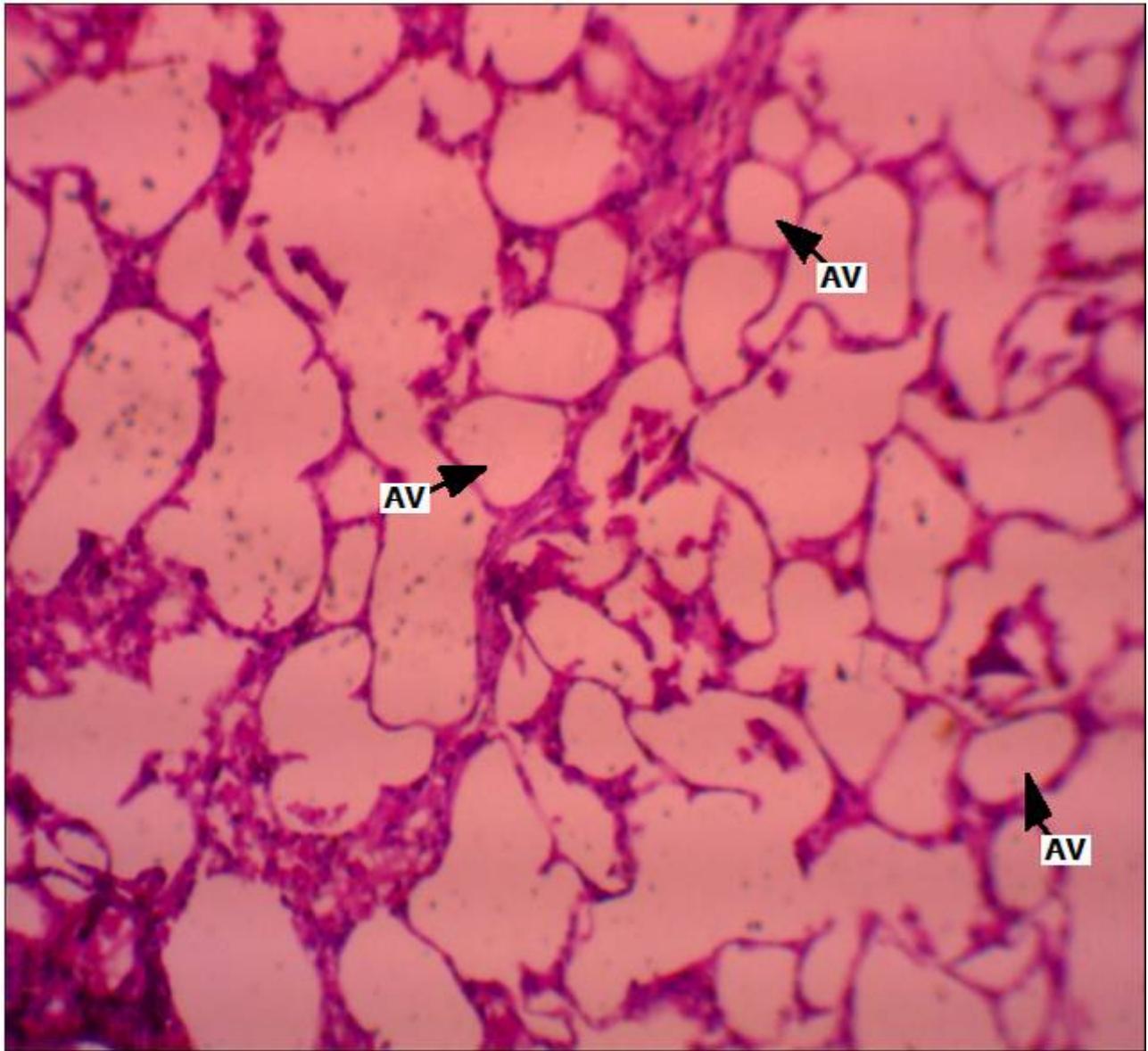


Fig. 3: Photomicrograph of test group C (400mg) rat lung tissue section (H&E x100) showing no necrotic changes but normal cytoarchitectural features of the lung including alveoli (AV) with intact walls.

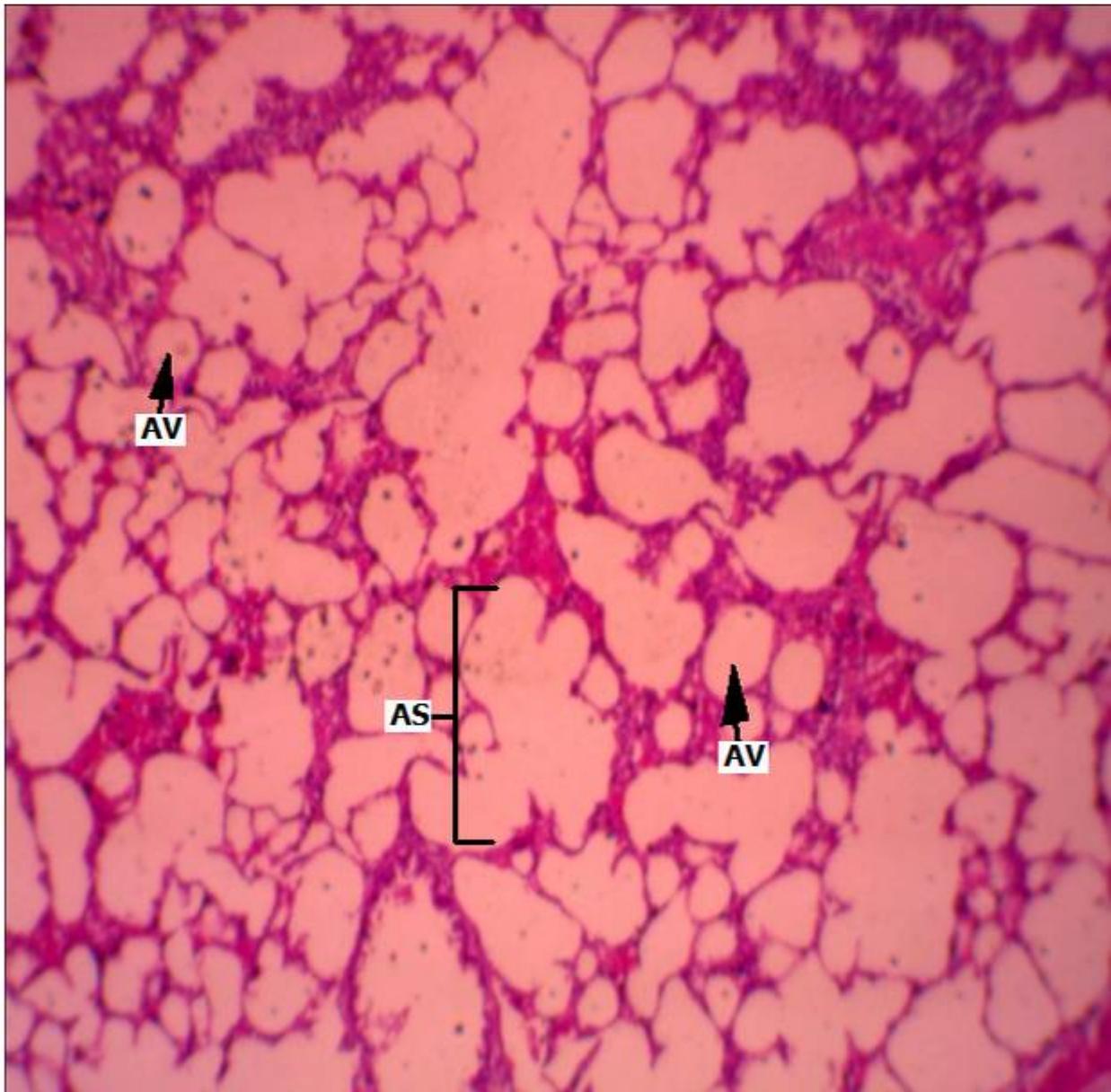


Fig. 4: Photomicrograph of test group D (600mg) rat lung tissue section (H&E x100) showing intact cytoarchitectural features of the lung with distinct alveoli (AV) and alveolar sacs (AS).



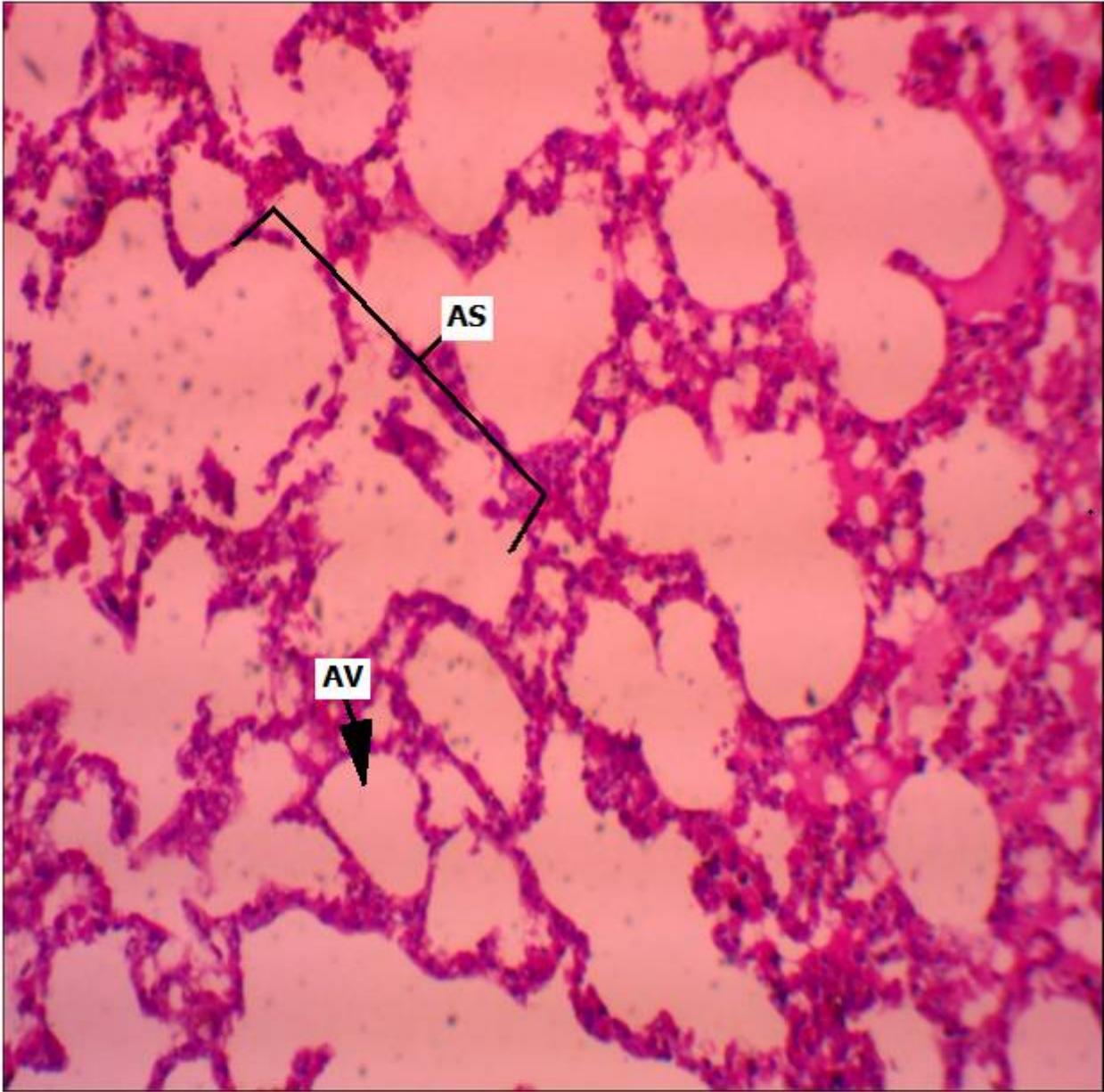


Fig. 5: Photomicrograph of test group E (800mg) rat lung tissue section (H&E x100) showing normal alveoli (AV) and alveolar sacs (AS).

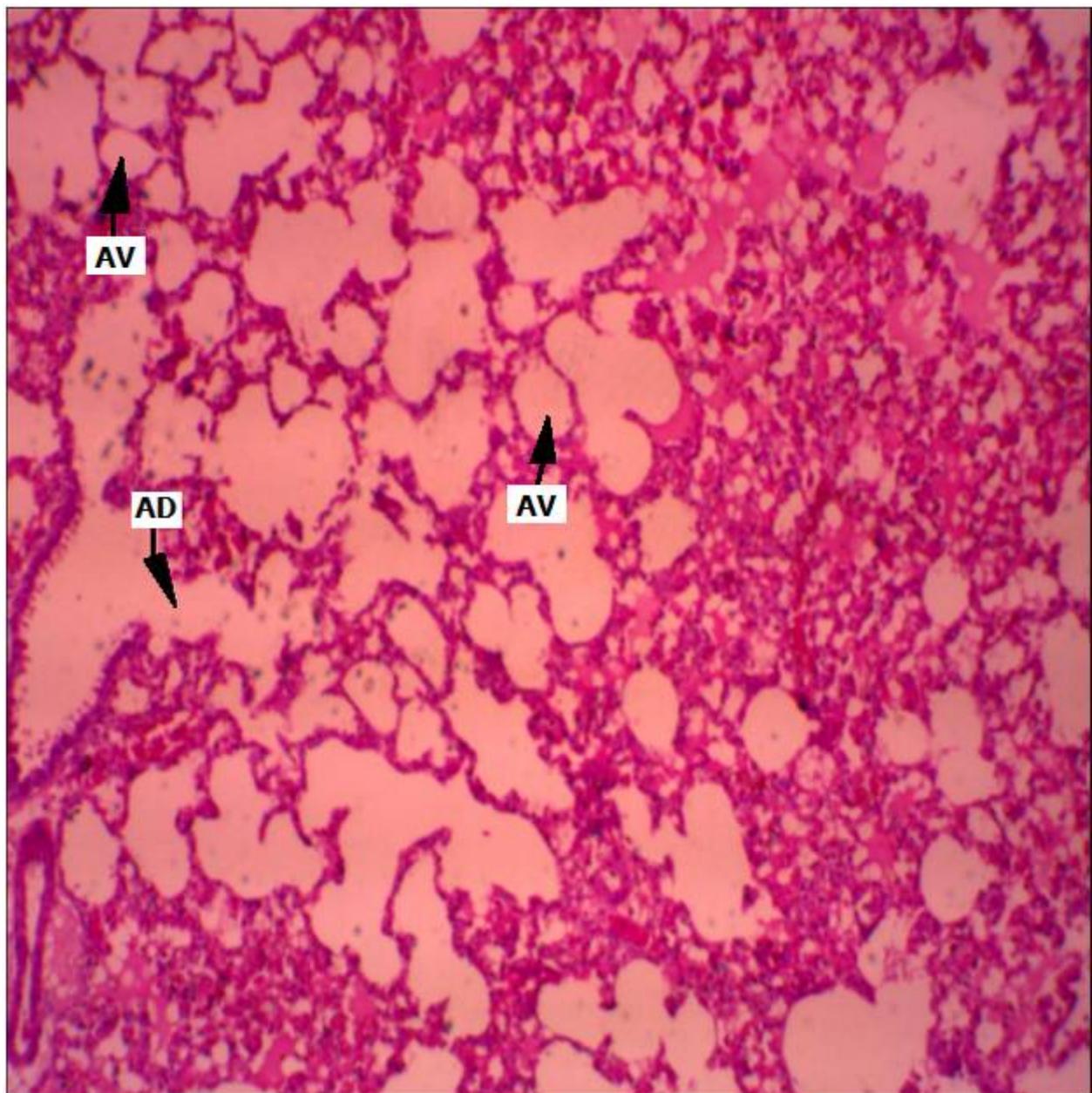


Fig. 6: Photomicrograph of test group F (1000mg) rat lung tissue section (H&E x100) showing normal fetures with intact alveoli (AV) and alveolar ducts (AD).



DISCUSSION

This study has shown that the ingestion of repeated doses of garlic powder, as administered to the rats in this study, did not induce any visible histological alteration in the lung tissue of the rats. The outcome was not even influenced by the fact that some of the administered doses were numerically greater than the documented LD₅₀ of 625.08mg/kg of garlic extract administered intraperitoneally (Nwanjo and Oze, 2006); though the difference in the route of administration (oral vs. intraperitoneal route) and the state of the substance of study (garlic powder vs. garlic extract), may account for the observed disparity.

Physiologically, the observed outcome is understandable, considering the exposure of the orally ingested garlic powder to the forces of digestion and absorption in the gastrointestinal tract, and the metabolic and detoxifying influence of the liver on the absorbed end-products, before being released into the open vascular system from which the lung tissue is supplied. Unfortunately, no reliable data on oral administered LD₅₀ was found as at the time of this study.

Although, the lung as a respiratory and excretory organ can be vulnerable to toxic xenobiotics, the findings by Daniel *et al.*, (2013) on the effect of *Allium sativum* and *Zingiber officiale* extracts in rats, showed that the plant extracts did not adversely affect the morphology of liver tissues in all the groups treated at both single and combined doses. They also observed that the effect of the extract at both single and combined doses suggested the non-toxic and non-deleterious effects on the liver tissue and hence, safe for consumption.

Safety tag notwithstanding, the assessments on bio-medically active and non-bio-medically active chemicals on animals, have been useful in predicting their toxicity in humans (Oduola *et al.*, 2007). More so, is evidence that laboratory findings on animals can be extrapolated towards predicting possible effects in humans, and that certain responses by humans are similar to those observed in experimental animals; despite obvious contradictions in some reported cases (Olson *et al.*, 2000).

CONCLUSION

Our findings therefore, suggest that a regulated consumption of garlic is safer; though more research is required to ascertain its effects at sub-cellular levels, while also undertaking a comprehensive characterization of the active substances in the various species of *Allium sativum*, in order to define its safety limits.

ACKNOWLEDGEMENT

We appreciate the Staff of the Histopathology Laboratory, College of Medical Sciences, Ambrose Alli University Ekpoma-Nigeria, for their support in the period of this research.

REFERENCES

- Daniel, E. E., Adelaiye, A.B., Lawan, A., Mohammed, A. and Tende, J. A. (2013): Effects of aqueous extracts of garlic (*Allium sativum*) and ginger (*Zingiber officiale*) on liver function profile in wistar rats. *Scientific Journal of Medical Science*; 2:(3).
- Daniel, Z., Maria, U. and Hopf, K. (2000): Domestication of plants in the Old world, third edition (Oxford: University Press), Pp. 197.
- Fleischauer, A.T and Arab, L. (2001): Garlic and cancer: a critical review of the epidemiologic Literature. *J. Nutrition*; 131: 1032-1040.
- Garlic-central (2011): Garlic health benefits. <http://www.garlic-central.com/garlic-health/html>
- Gualtiero, S. and Stanley, S. (1990): Guide to Herbs and Spices. Simon and Schuster, Inc.



- Kodera, Y., Matuura, H., Yoshida, S., Sumida, T., Itakura, Y., Fuwa, T. and Nishino, H. (1998): Allixin: a stress compound from garlic. Retrieved January 30,2009.
- Kojuri, L., Vosoughi, A.R. and Akrami, M. (2007): Effects of *Anethum graveolens* and garlic on lipid profile in hyperlipidemic patients. *Lipids Health Dis*: 1 (6):5
- Nagakawa, S., Masamoto, K., Sumiyoshi, H., Kunihiro, K. and Fuwa, T. (1995): Effect of raw and extracted aged garlic on the growth of young rats and their organ after peroral administration. *J. Toxicol. Sci.*; 5: 91-112.
- Nwanjo, H. and Oze, G. (2006): Antiarrhythmic and Anti Anti-Hyperlipidaemic Potentials of Aqueous Garlic Extract IN Hypercholesterolaemic rats. *The Internet Journal of Nutrition and Wellness*; 3(2).
- Oduola, T, Popoola, G.B., Avwioro, O.G., Oduola, T.A., Ademosun ,A.A. and Lawal, M.O. (2007): Use of *Jatropha gossypifolia* stem latex as a haemostatic agent: how safe is it? *J. Med. Plants Res.* 1(1): 014-017.
- Olson, H., Betton, H., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., *et al.*(2000): Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. *Regulatory Toxicology and Pharmacology*. Vol 32(1) :56-57.
- Paul, B. (1996): *The healing Power of Garlic* . Published by Prima Publishing company.
- World Gazetteer (2007): Population of Cities, news, divisions. <http://worldgazetteer.com/ng.php>. Retrieved on 23/05/2008

AUTHOR'S CONTRIBUTIONS

IDEHEN, I.C ; Chief researcher, laboratory supervisor and content analyst.
 DIC-IJIEWERE, O.E.; Resource supervisor, content and result analyst
 ALARI-ENOMA, O.; Content, Sampling/ Laboratory analyst
 AIRHOMWANBOR, K.O.; Resource and content analyst
 EIDANGBE, A.P.; Co-researcher
 UWUIGBE, M.; Contributor
 NWAOPARA, A.O; Co-researcher

