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Histological Evaluation of the Sub-chronic Toxicity of Methanol Extract of *Tetracarpidium conophorum* (African Walnut) Seeds in Wistar Albino Rats

*[1]Oriakhi, K, [2]Uadia, P.O. and [3]Eze, G.I
[1]Department of Medical Biochemistry, School of Basic Medical Sciences, College of Medical sciences, University of Benin, Benin City, Edo State, Nigeria
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria
[3]Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State. Nigeria

Abstract
The purpose of this study was to examine the histopathological effect of methanol extract of Tetracarpidium conophorum seeds on the sub chronic toxicity in Wistar rats. Twenty female rats were divided into 4 groups of 5 rats each; the animals were fed rats mash and given water orally ad libitum. Group I (control) was given distilled water only, while rats in group II, III and IV were administered methanol extract of T. conophorum seeds at different concentration of 500, 1000, and 2000 mg/kg body weight for 30 days. The results show that, there was no death observed and no distortions in the architecture of the various organs (Liver, Spleen, Kidney, Heart and Lungs) when administered various doses of the extract, rather the extract was beneficial to the rats by activating lymphoid cells. It could be concluded that the extract may possess immunogenic properties.

Keywords: Toxicity, *Tetracarpidium conophorum*, Sub chronic toxicity, Histopathological

Introduction
Herbal medicine has become an important component of health care delivery system in many countries around the world. Currently, about 80% of world population depends on herbs for the treatment of various diseases (1). This is because medicinal plants are rich sources of compounds that possess therapeutic effects which are easily accessed and affordable especially by people in developing countries. However herbal preparations are usually not screened for its toxicity before consumption, which may be deleterious to humans (2). The growing interest in herbal medicine therefore demands toxicity risk assessment of the various indigenous preparations used in the treatment of diseases (3).

Tetracarpidium conophorum (African walnut) belongs to the family of Euphorbiaceae and it is commonly known in Southern Nigeria as ukpa (Igbo), in Western Nigeria as awusa or asala (Yoruba) and okhue in Bini. It is a perennial climber found in the moist forest zone of sub- Sahara Africa (4). The leaves are globrous, ovate, long and margin toothed. The bases of the leaves are broaded and rounded up to 15 inches with slender petioles that are up to 2 inches long. The fruits are four winged, ridged between wings and up to 3 inches in diameter. African walnut has a bitter taste upon drinking water and can be eaten raw or cooked (5, 6). This plant possesses multiple medicinal properties such as antioxidant and immunostimulatory properties (7), antifertility, antimicrobial (8), and anticancer (9). Therefore the purpose of this study is to examine the histopathological effect of methanol extract of *T. conophorum* on the sub chronic toxicity of the extract in Wistar albino rats.

Materials and Methods
Collection of Plant Materials
The seeds of *T. conophorum* used in this study were obtained from open forest at Ovia North East Local government area, Edo state, Nigeria. The fresh walnut seeds were identified by Prof. M.E. Osawaru and authenticated by Professor MacDonald Idu both of the Department of Plant Biology and Biotechnology of the University of Benin, Benin City, Nigeria. Herbarium specimen (voucher number UBHe0153) was deposited at the Herbarium of the University of Benin.

Preparation of Plant Extract
The seeds were rinsed properly, de-shelled, cut into pieces, and shade dried. The dried samples were then pulverized into powder and stored in an air-tight container. *T. conophorum* seed powder (1 kg) was extracted in 5000 mL of absolute methanol at room temperature for 72 hours. The samples were filtered with Whatman No. 50 filter paper and the filtrate evaporated to dryness with a rotary evaporator (RE 300, Bibby Scientific, UK) to give 320 g corresponding to a percentage yield of 32 %. The resultant yield was stored in an air-tight bottle and kept in the refrigerator maintained at 4^{o}C.

*Corresponding Author's E-mail: Kelly.oriakhi @uniben.edu

Animals

Eight to ten week old female Wistar rats of average weight 140±10 g were used in this study. The rats were obtained from the animal house in the department of Biochemistry, University of Benin. Prior to the study, they were acclimatized to laboratory conditions for seven days. The rats were fed with rat chow and allowed free access to clean drinking water. All animal experiments were conducted according to NIH guidelines (10).

Sub-chronic Toxicity Study

A total of twenty mature female albino rats were used in this study; these were divided into four groups of five rats each. Three of the groups were given 500, 1000 and 2000 mg/kg body weight of the methanol extract of *T. conophorum* seeds orally, for 30 days, while the control group received distilled water only orally for 30 days. After 30 days of exposure, fasting blood was collected via cardiac puncture for histological assessment.

Histological Assessment

Kidney, liver, heart, lungs and spleen from rats of all the groups were fixed in 10 % formaldehyde, dehydrated in graded alcohol and embedded in paraffin. Fine sections were obtained, mounted on glass slides and counter-stained with hematoxylin-eosin (H&E) for light microscopic analyses. The slides were coded and examined by a Histopathologist who was ignorant of the treatment groups.

Statistical Analysis: Results were expressed as Mean ± SEM. Statistical analyses was carried out by one-way ANOVA (Prism 6.0); data was further subjected to dunnett's post host test and differences between treated groups and control accepted as significant at $p<0.05$.

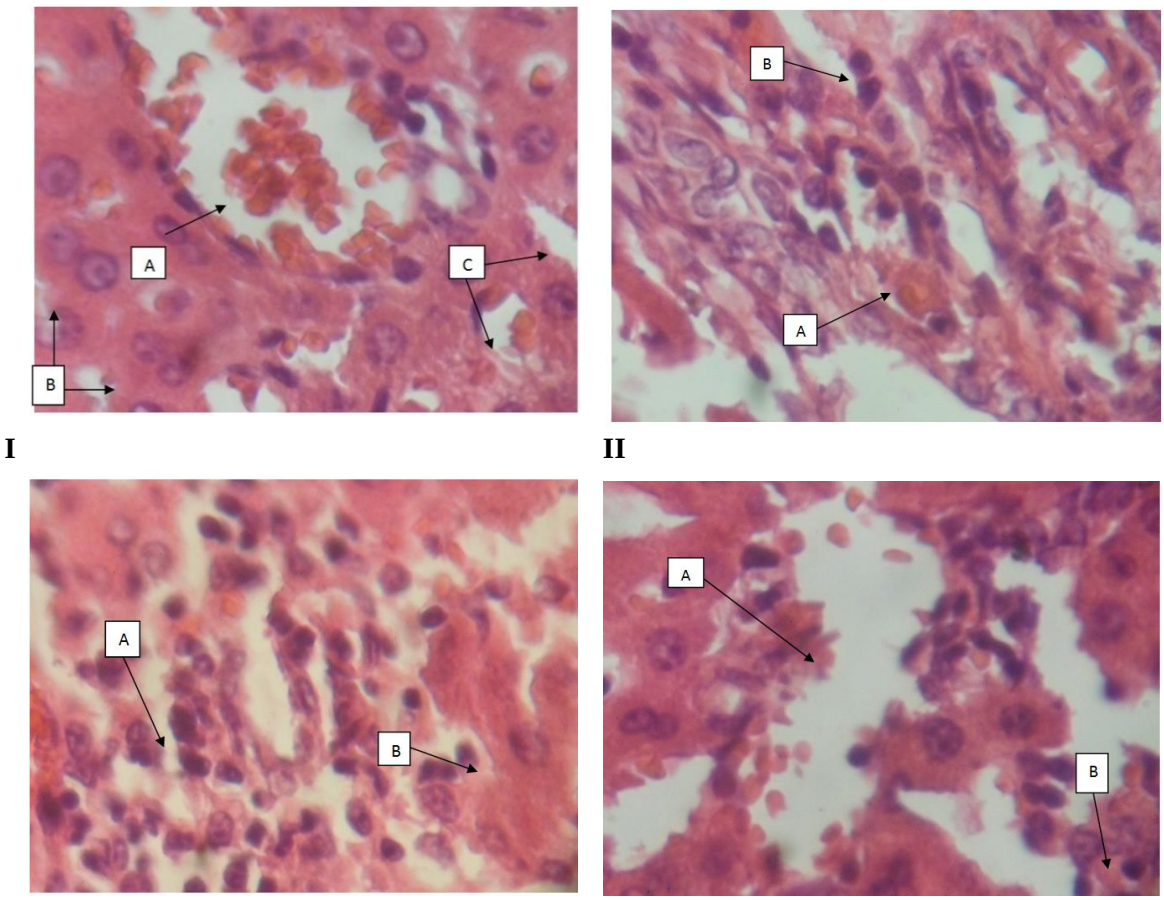
Results and Discussion

Herbal medicines are widely perceived by the public as being natural and free from side effects. Most people believe that herbal medicines have no side effects or any potential risks due to their natural origins and are often considered as food supplements and not drugs. Medicinal herbs are usually self-prescribed by the consumers and there is a lack of control and review in terms of dose, manner, and frequency of administration. The chemicals in medicinal herbs may be natural to the plant, but they are not natural to the human body.

This study focused on the herbal plant of *T. conophorum* which has extensively been used among the African community for its medicinal properties. The increased use of this plant has resulted in concerns for both the efficacy and safety of the product. In the present study, histopathology evaluation of methanol extract of various doses (500, 1000 and 2000 mg/kg body weight) of *T. conophorum* seeds on the liver, kidney, spleen, lung and heart was carried out after it was administered daily for thirty days to the Wistar rats. Results indicated that the extract did not adversely affect the morphology of the rats' organs; rather there was an improvement in the immunity of the animals by the extract as shown in the histology results. This finding is in agreement with the observation of Uadia *et al*. (2012) which stated that methanol extract of *T. conophorum* seeds increased white blood cells and lymphocytes. However, the figures below show the effect of methanol extract of *T. conophorum* seed on liver, kidney, spleen, lung and heart and the photomicrograph of the various organ sections.

Effect of methanol extract of *T. conophorum* seeds on the liver of Wistar Rats

Photomicrographs of the hepatic morphology of all the treatment groups are shown in figure 1. Liver sections from rats in group I (Control) appear normal without any visible lesion while groups II to IV which were administered 500, 1000 and 2000 mg/kg body weight of *T. conophorum* seeds showed mild portal congestion and mild periportal lymphocytosis. However, the sinusoidal kupffer cells were activated at doses 1000 and 2000 mg/kg body weight of the extract. Since kupffer cells are resident hepatic macrophages, their activated state due to administration of *T. conophorum* seed is also an indication of the ability of this seed to enhance the immune status of the animals.



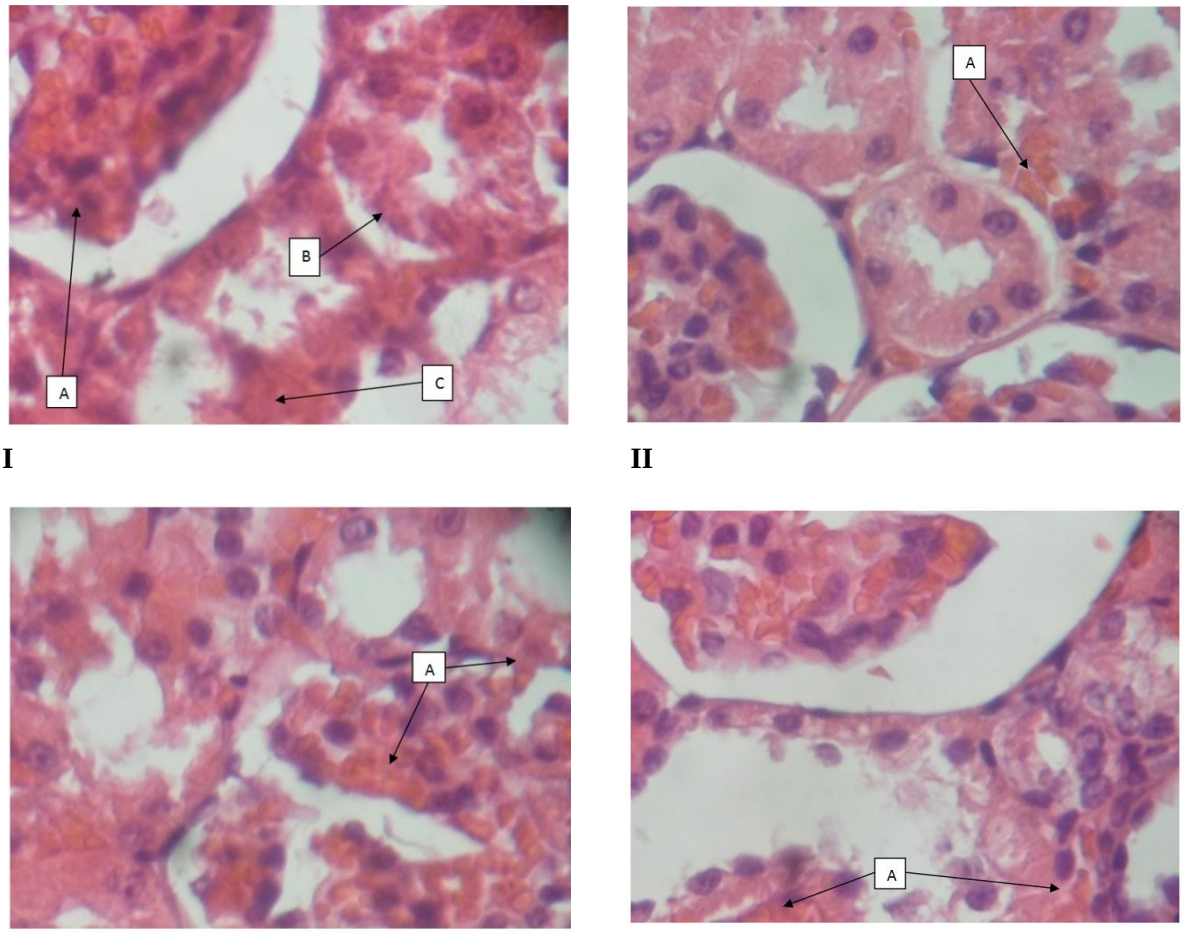
I **II**

III **IV**

Figure 1: ***I*** *Control): Rat liver composed of portal vein A, hepatocytes B and sinusoids C. Intact lobular architecture and normal hepatocytes with no cytoplasmic inclusions or collections. The portal tracts, central veins, and capsules are normal with no evidence of adhesion or inflammation;* ***II*** *Rat liver given 500 mg/kg T. conophorum for 30 days showing mild portal congestion A and mild periportal lymphocytosis B;* ***III*** *Rat liver given 1000 mg/kg T. conophorum showing mild periportal lymphocytosis A and sinusoidal kupffer cell activation B;* ***IV*** *Rat liver given 2000mg/kg T. Conophorum showing mild sinusoidal congestion and dilatation A and kupffer cell activation B (H&E x 400)*

Effect of methanol extract of *T. conophorum* seeds on the kidney of Wistar Rats

Photomicrographs of the kidney morphology of all the treatment groups are shown in figure 2. Group I(Control) shows preserved medullary and cortical architecture as well as proximal and convoluted tubules with an intervening loop of Henle's, while groups II to IV at the various doses of 500, 1000 and 2000 mg/kg body weight of the extract showed mild interstitial congestion.



I **II**

III **IV**

***Figure 2**: **I** Control: Rat kidney composed of glomerulus A, tubules B and interstitial space C; **II** Rat kidney given 500 mg/kg T. conophorum showing mild interstitial congestion A; **III** Rat kidney given 1000 mg/kg T. Conophorum showing moderate interstitial congestion A; **IV** Rat kidney given 2000 mg/kg T. conophorum showing moderate interstitial congestion A (H&E x 400)*

Effect of methanol extract of *T. conophorum* seeds on the spleen of Wistar Rats

Photomicrographs showing the spleen morphology of all the treatment groups are presented in figure 3. Spleen sections from rats in group I (Control) appear normal without any visible lesion while groups II to IV which were administered 500, 1000 and 2000 mg/kg body weight of methanol extract of *T. conophorum* seeds showed mild activation of lymphoid aggregate and histiocytes. Spleen is one of the organs of the immune system and the activation of the lymphoid aggregate by the extract correlates well with the lymphocytosis observed in the liver.

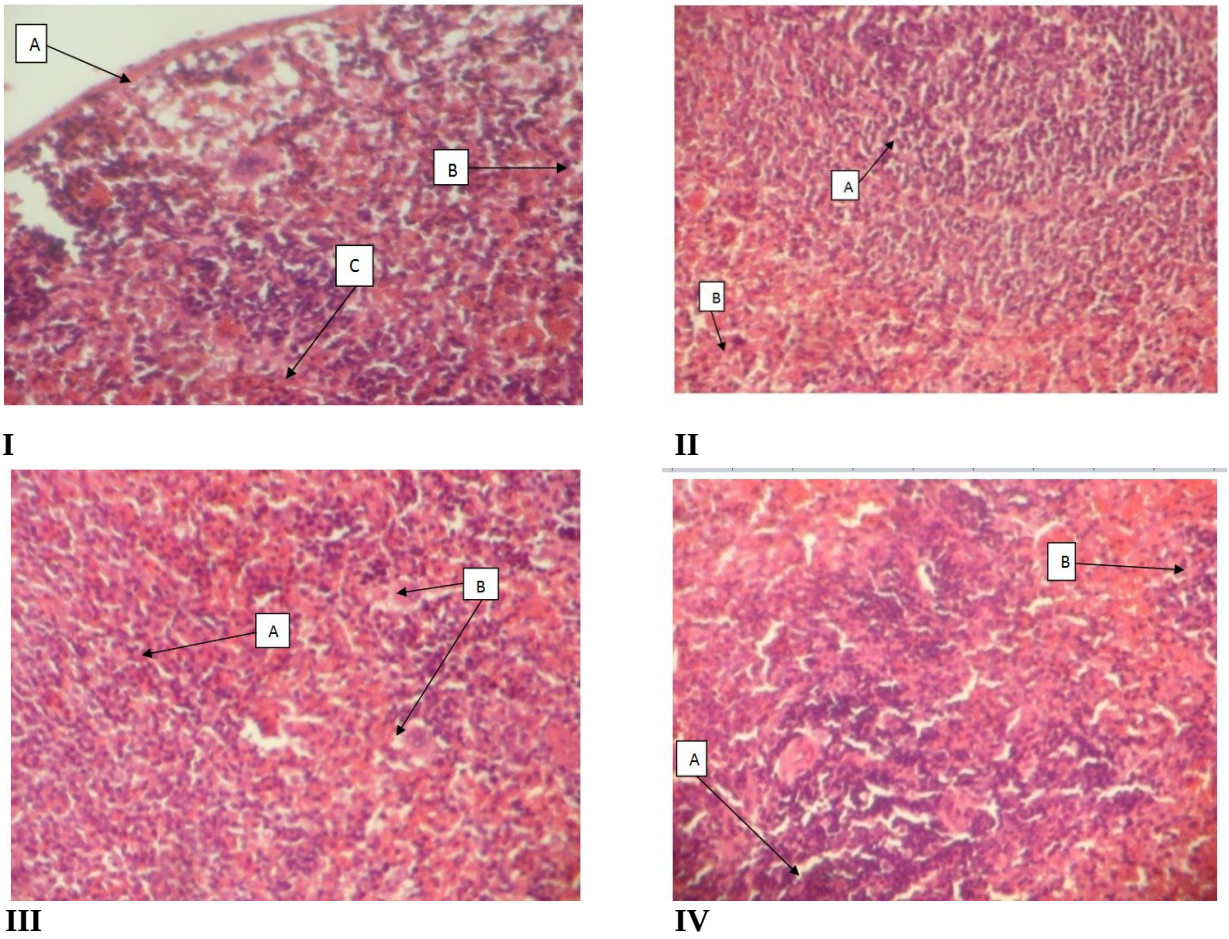


***Figure 3**: **I** (Control): **I** Rat spleen composed of capsule A, red pulp B and white pulp C; **II** Rat spleen given 500mg/kg T. conophorum for 28 days showing mild activation of lymphoid aggregates A and sinus histocytes B; **III** Rat spleen given 1000 mg/kg T. conophorum for 30 days shows mild lymphoid activation A and moderate histiocytosis B; **IV** Rat spleen given 2000 mg/kg T. conophorum showing mild lymphoid activation A and histiocytosis B (H&E x 100)*

Effect of methanol extract of *T. conophorum* seeds on the heart of Wistar Rats

Photomicrographs showing the heart morphology of all the treatment groups are presented in figure 4. Heart sections from rats in group I (Control) appear normal without any visible lesion while group II which was administered 500 mg/kg body weight of the extract showed mild interstitial congestion i.e. the blood vessels in the interstitial spaces are mildly congested. The extract is vasogenic, acting on the blood vessels causes more blood to flow to the heart. Component of the heart appear unremarkable. However when the 1000 and 2000 mg/kg body weight of the extract was administered to groups III and IV respectively, there was also mild interstitial fluid which could be as a result of the preservative process or increased congestion.

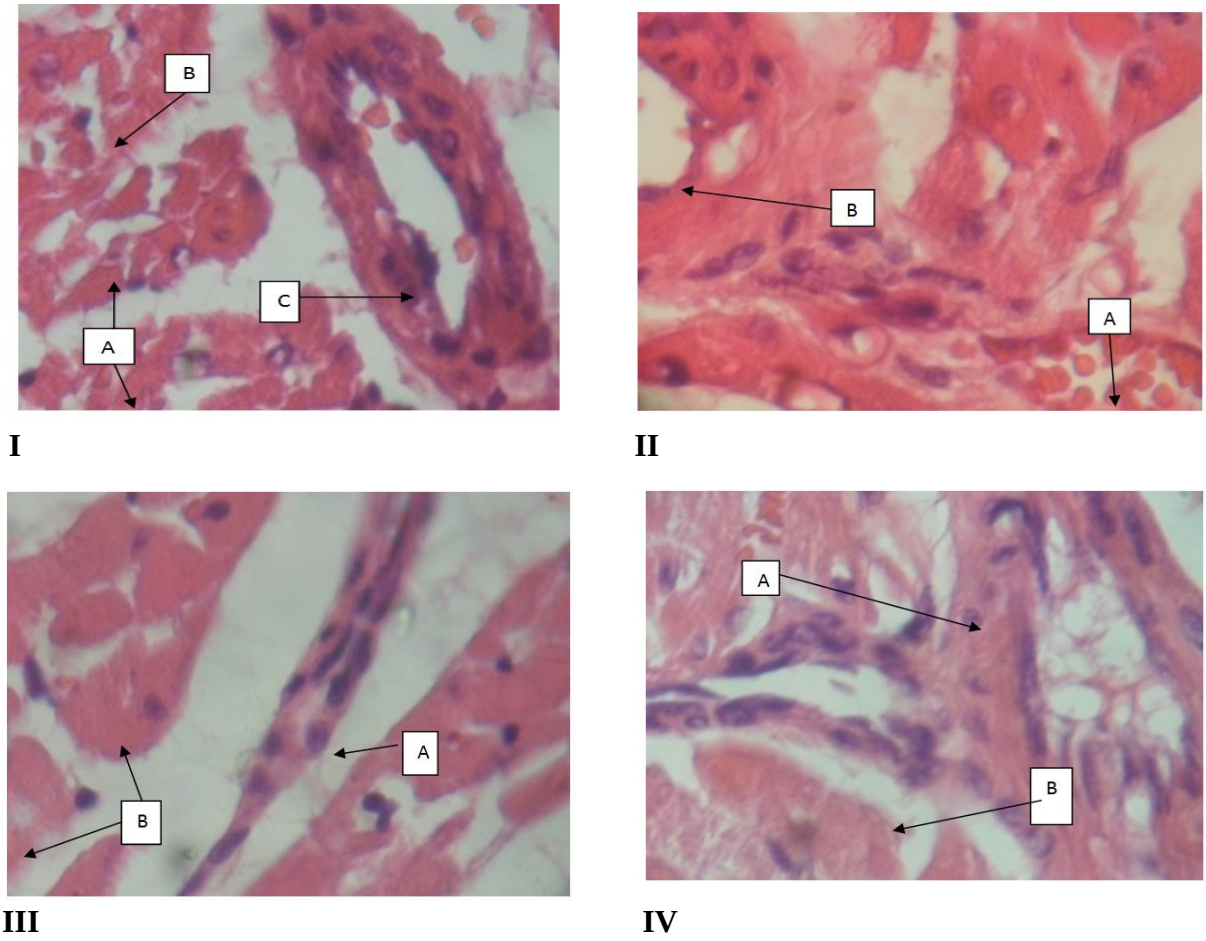


Figure 4*(Control):* ***I*** *Rat heart composed of bundles of myocardiac fibres A separated by interstitial space B and coronary artery C;* ***II*** *Rat heart given 500mg/kg T. conophorum for 30 days showing mild interstitial congestion A, coronary artery B; Rat heart given 1000mg/kg T. conophorum for 30 days showing mild interstitial oedema A, bundles of myocardiac fibres B; Rat heart given 2000mg/kg T. conophorum for 30 days showing mild coronary vascular hypertrophy A, mild interstitial oedema B (H&E x 400).*

Effect of methanol extract of *T. conophorum* seeds on the lungs of Wistar Rats

Photomicrographs showing the lung morphology of all the treatment groups are presented in Figure 5. Lung sections from rats in group I (Control) appear normal without any visible lesion while in groups II, III and IV, the lymphoid aggregate were activated when administered 500, 1000 and 2000 mg/kg body weight of the extract respectively. However the extract was more immunogenic at dose 500 mg/kg body weight when compared to other doses.

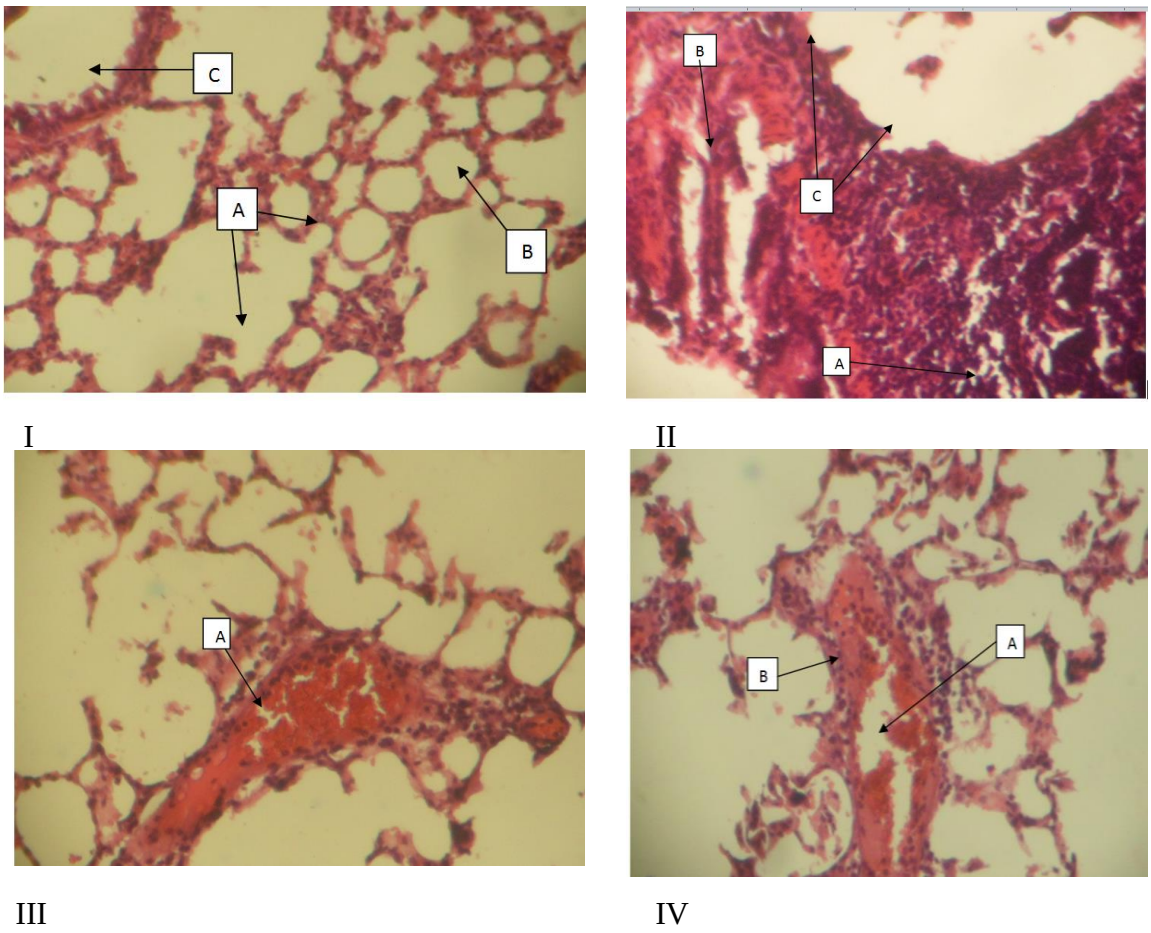


***Figure 5*: *I* (Control): Rat lungs composed of alveolar sacs A, interstitial space B and terminal bronchiole C; *II* Rat lungs given 500 mg/kg T. conophorum for 30 days showing florid activation of bronchiolo alveolar lymphoid aggregates A, terminal bronchiole B and alveoli C; *III* Rat lungs given 1000 mg/kg T. conophorum for 30 days showing mild interstitial congestion A; *IV* Rat lungs given 2000 mg/kg T. conophorum for 30 days showing mild vascular congestion A and hypertrophy B (H&E x 100)**

Conclusion

The evaluation of the possible effects of methanol extract of *Tetracarpidium conophorum* seeds on the various organs did not record any histological distortion. However the extract could be beneficial because it has the ability to improve the immunity of the rats.

Acknowledgement

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Conflict of Interest

The author declare no conflict of interest

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