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Histological effects of aqueous extract of *Cymbopogon citratus* leaf on *Areca catechu*-induced esophageal injury in adult Wistar rats

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Abstract:

BACKGROUND: *Cymbopogon citratus* (lemongrass) has been reported to possess antimicrobial, antioxidant, antimutagenic, and anticarcinogenic properties, as well as the ability to modify gene transcription. The seeds of *Areca catechu* contain carcinogenic compounds.

AIM: This study, therefore, evaluated the effects of aqueous extract of *C. citratus* on *A. catechu*-induced esophageal injury in adult Wistar rats.

MATERIALS AND METHODS: A total of thirty rats weighing between 180 and 250 g were obtained from the laboratory animal facility of the Department of Anatomy, University of Benin, Benin city. The animals were randomly assigned to six groups of five animals each. Group A served as control group and received food and distilled water only, Group B received 1500 mg/kg body weight (bw) of *A. catechu* only, Group C received 1500 mg/kg bw of *C. citratus* only, Group D received 1500 mg/kg bw of *A. catechu* and 1000 mg/kg bw *C. citratus,* Group E received 1500 mg/kg bw of *A. catechu* and 2000 mg/kg of *C. citratus,* while Group F received 1500 mg/kg bw of *A. catechu* and 3000 mg/kg bw of *C. citratus.* The administration of extracts was commenced through oral gavage for 4 weeks. The rats were sacrificed, and appropriate histological procedures were carried out.

RESULTS: Epithelial proliferation in the mucosa of the esophagus was caused by A. *catechu,* whereas C. *citratus* attenuated this lesion, as well as activating the immune system.

CONCLUSION: Findings from the histological results showed that *C. citratus* protected the esophagus from the damages induced by *A. catechu.*

Keywords:

Areca catechu, Cymbopogbon citratus, esophagus, lymphocytes

Introduction

The use of herbs as medicine is a very common practice in developing countries, particularly in rural settlements. Over the last decade, an increase in the medicinal use of plants has been observed in urban areas of developed countries (Harnack *et al*., 2001).

A large majority of herbal plants possess pharmacological properties, which have made them curatives for various diseases. According to the World Health Organization (De Silva, 1997), about 80% of the population in many third world countries still use traditional medicine as their source of primary health care due to a paucity of wherewithal and lack of access to modern medicine. Plants are also used extensively to relieve digestive dysfunctions. Several studies have shown that antioxidants present in these plants preserve an adequate function of the digestive system. Therefore, the protection of this system afforded by dietary antioxidant supplementation

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plays an important role in order to achieve a healthy living (Knight, 2000).

The safety of many of the herbal drugs is only relative, but the population feel more at ease with their usage because of their long and widespread usage and also their familiarity with these plants (Nantia *et al.*, 2009). Consequently, one of such medicinal plants worthy of note is *Cymbopogon citratus*. *C. citratus* was, in earlier times, named *Andropogon citratus* by De Candolle and later re-classified by Otto Stapf. It is a member of the Poaceae family that has about 500 genus and 8000 herb species, commonly known as grasses (Barbosa *et al.*, 2008). *C. citratus* is popularly known as lemongrass, and has been cultivated for several years for medicinal purposes in West Africa. It has a maximum height of about 1.8 m and its leaves are about 1.9 cm wide, covered with a whitish bloom. In Ghana, Liberia, Nigeria, Sierra Leone, and Guinea Bissau, the use of its fragrant oil cuts across diversities. Some of its traditional uses include the utilization of its fragrant leaves, which contain the volatile oil, in the form of tea as an antipyretic, while the roots are used as chewing sticks or modified toothbrushes. In certain climes, it is used as a panacea for mental illness. It is also reported to be an antifungal, antioxidant, and deodorizing agent. In combination with other herbs, it is commonly used as a cure for malaria (Onawunmi *et al*., 1984; Gbile, 1986).

Betel nut (*Areca catechu*) is a slender, single-trunked palm that can grow up to 30 m (100 ft). The Yorubas call it Ekuọ Oyinbo, the Hausas call it Kwakwa Moinja Nbature, the Igbos call it Aku Ndi Ọcha, while the Binis call it Ikpedin Okhe Ebo (Adediji *et al*., 2015). It is cultivated from East Africa and the Arabian Peninsula across tropical Asia and Indonesia to the central Pacific and New Guinea. The nut is chewed as a stimulant masticatory by 5% of the world's population, making it more popular than chewing gum, but not as popular as tobacco (Staples and Bevacqua, 2006).

However, the mastication of *A. catechu* has been revealed to be one of the major risk factors of hepatocarcinoma and oropharyngeal and esophageal cancers. Arecoline, the main *Areca* alkaloid of the betel nut, is reported to have cytotoxic, genotoxic, and mutagenic effects in various cells (Chandak *et al*., 2013). It shows a strong correlation to the incidence of oral submucosal fibrosis, leukoplakia, and oral cancer, and has also been found to impose toxic manifestations in immune, hepatic, and other defense systems of the recipient (Dasgupta *et al*., 2006).

Because *A. catechu* has been linked to esophageal cancer, it has become necessary to study the possible effect of a well-known antioxidant species on *A. catechu*-induced esophageal injury.

Materials and Methods

Plant materials

A. catechu nuts were collected from the Department of Anatomy, in the University of Benin, while the plant *C. citratus* was collected from Botanical Gardens, Sapele road, Benin City, and were subsequently identified by a curator in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State.

Extract

After the collection of plants, they were air-dried for 2 weeks, pulverized, and an aqueous extract was obtained according to standard methods (Farooqi and Ahmad, 2017) [Table 1].

Animals

A total of thirty adult Wistar rats of either sex were used for the experiment. The animals were purchased from the Animal House of the Department of Anatomy, University of Benin, Benin City, Edo State, and were maintained according to standard laboratory practices.

Experimental design

The Wistar rats weighing between 140 g and 160 g were randomly assigned to six groups of five animals each. Food and water were provided across all groups, *ad libitum*. Ethical approval was given by the Research and Ethics committee of the School of Basic Medical Sciences, University of Benin, Benin City.

Histopathology

The esophagi were excised and stored in bottles containing Bouin's fluid, ready for histology. The

Table 1: Amount of plant material and volume of solvent for the preparation of extracts

Plant	Solvent	Weight (g)	Volume (ml)
Areca catechu	Distilled water	20	200
Cymbopogon citratus	Distilled water	20	200

Table 2: Treatment regimen

Groups	Dosage
Group A	Rat feed and distilled water only
Group B	*A. catechu* only (1500 mg/kg of body weight)
Group C	*C. citratus* only (1500 mg/kg of body weight)
Group D	*A. catechu* (1500 mg/kg of body weight) and low dose of *C. citratus* (1000 mg/kg of body weight) simultaneously
Group E	*A. catechu* (1500 mg/kg of body weight) and moderate dose of *C. citratus* (2000 mg/kg of body weight) simultaneously
Group F	*A. catechu* (1500 mg/kg of body weight) and high dose of *C. citratus* (3000 mg/kg of body weight) simultaneously

A. catechu - Areca catechu, C. citratus - Cymbopogon citratus

procedures were carried out according to the standard methods of Drury *et al*. (1976) Photomicrographic plates of the desired sections were taken, using a standard photomicrography setup.

Results

Figures 1 and 2 show the esophagus of the Wistar rat of the control group which is a healthy esophagus comprising stratified squamous epithelia, lamina propria, muscularis propria, submucosa, and muscularis propria.

However, after the treatment of the esophagus of the Wistar rats in Group B with the aqueous extract of *A. catechu,* epithelial proliferation in the mucosa of the esophagus and lesions and mild activation of the immune system (as observed by the activation of lymphocyte population in the submucosa) were detected [Figures 3-6]. These are all manifestations of esophageal damage.

Figures 7 and 8 show Wistar rats' esophagus treated only with *C. citratus* (1500 mg/kg body weight), at low magnification and high magnification, respectively,

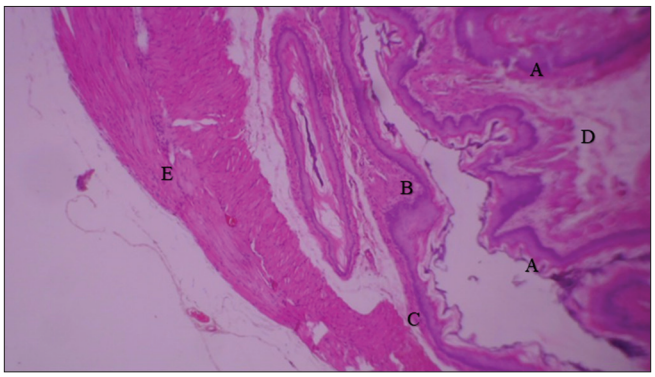

Figure 1: Control; rat esophagus comprised of A: Stratified squamous epithelial lining, B: Lamina propria, C: Muscularis mucosa, D: Submucosa, and E: Muscularis propria (H and E, ×40)

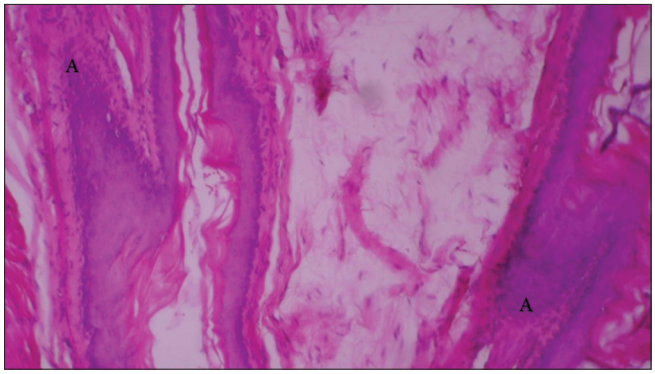

Figure 3: Rat esophagus given 1500 mg/kg *Areca catechu* only, showing A: Focal proliferation of the squamous epithelium in the lamina propria (H and E, ×100)

showing mild infiltrates of lymphocytes. The plate also showed no pathology.

Figures 9 and 10 show rat esophagus treated with *A. catechu* plus low dose of *C. citratus* which demonstrate normal epithelial lining, bereft of the pathological conditions as shown in Figures 3-6 of Group B. In the groups administered with medium dose and high dose of *C. citratus* (Group E and Group F) to combat *A. catechu*-induced esophageal damage, similar results were observed [Figures 11-14]. The histoarchitecture of the esophagus was compared favorably with that of the control group, although better histological results were achieved in the groups administered with low dose (Group D) and medium dose (Group E), as evidenced by microscopical assessment.

Discussion

The focal point of this study was the histological effect of the esophageoprotective ability of *C. citratus* on *A. catechu*-induced esophageal injury. *A. catechu* has been demonstrated to elicit oropharyngeal and esophagus cancers (Dasgupta *et al.*, 2006), while on the other hand, *C. citratus* has been traditionally used to treat gastrointestinal disorders due to its antioxidant and chemoprotective properties (Devi *et al*., 2011).

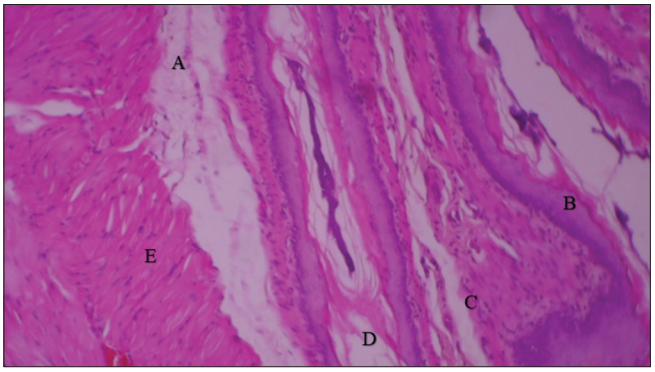

Figure 2: Higher magnification: A, B, C, D, and E (H and E, ×100)

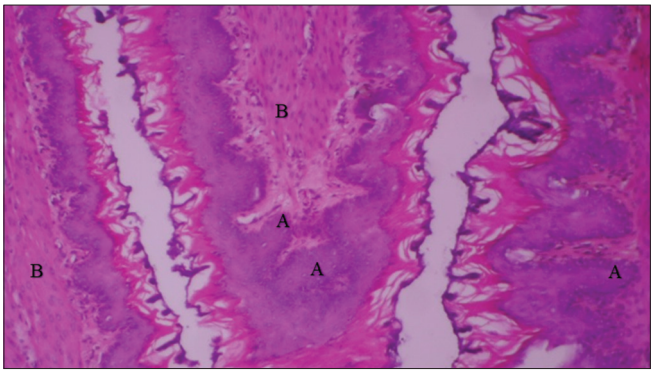

Figure 4: Rat esophagus given 1500 mg/kg *Areca catechu* only, showing A: Finger-like projection of the epithelium into the B: Highly cellular connective tissue stroma of the subepithelium (H and E, ×100)

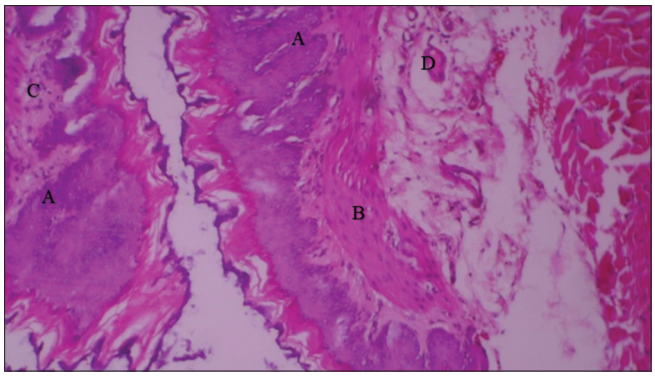

Figure 5: Rat esophagus given 1500 mg/kg *Areca catechu* only, showing A: Marked papillary projection of the epithelium into the B: Thick collagenous stroma of the subepithelial zone, C: Mild stromal infiltrates of lymphocytes, and D: Mild submucosal congestion (H and E, ×100)

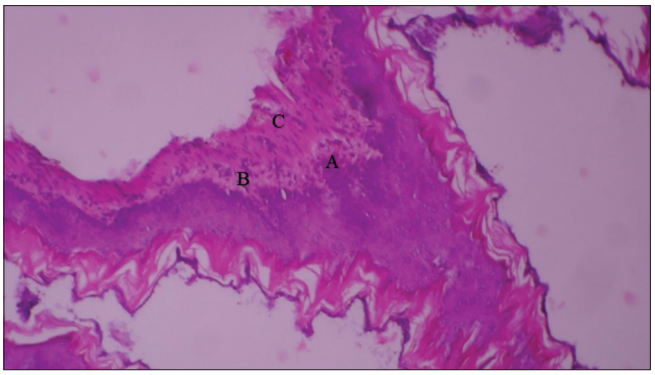

Figure 6: Same group showing, A: Focal epithelial proliferation and B: Moderate infiltrates of lymphocytes into the C: Highly cellular subepithelial zone (H and E, ×100)

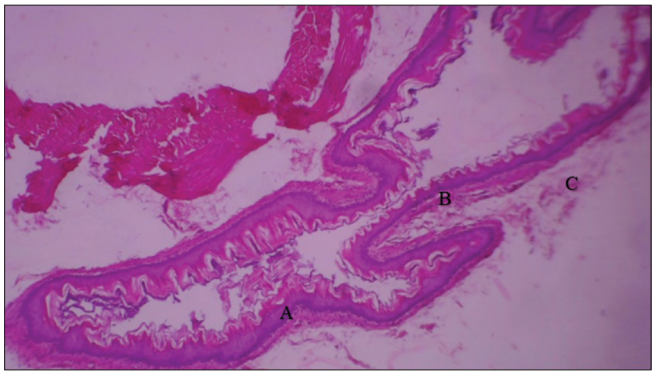

Figure 7: Rat esophagus given 1500 mg/kg *Cymbopogon citratus* only showing A: Normal epithelial lining, B: Mild infiltrates of lymphocytes in the lamina propria and C: Mild active vascular congestion (H and E, ×40)

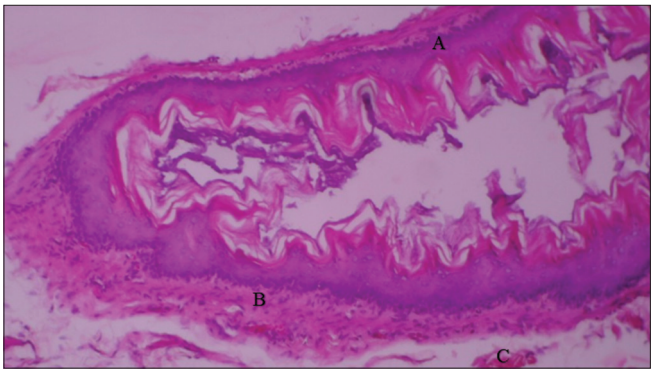

Figure 8: Higher magnification: A, B, and C (H and E, ×100)

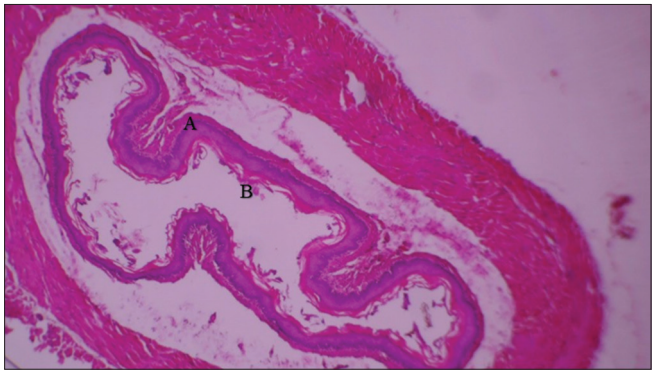

Figure 9: Rat esophagus given *Areca catechu* + 1000 mg/kg *Cymbopogon citratus* showing A: Normal epithelial lining and B: Mild active submucosal congestion (H and E, ×40)

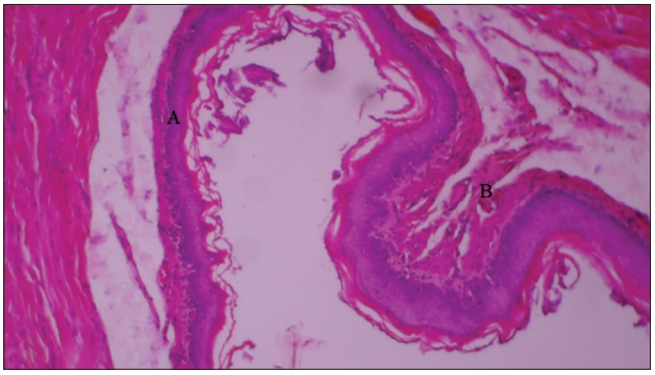

Figure 10: Higher magnification: A and B (H and E, ×100)

Esophageal carcinoma ranks among the ten most frequent malignancies worldwide (Jemal *et al.*, 2005). The relationship between *A. catechu* and the incidence of esophageal cancer has been reported as far back as 1966 (Dunham, 1968). Findings by Jeng *et al.* reported that components of *A. catechu* induced keratinocyte inflammation by stimulating the production of prostaglandin E2, tumor necrosis factor-α, and interleukin-6 in primary cultured gingival keratinocytes and KB oral carcinoma cells. Furthermore, the International Agency for Research on Cancer review concluded that areca nut is carcinogenic in humans and that it is linked to cancers of the oral cavity, pharynx, esophagus, liver and biliary tracts, and uterus (Bhisey *et al.*, 2004).

In the present study, *A. catechu* was found to be mitogenic, as demonstrated by papillomatous proliferation of the epithelia and development of lesions in the esophagi of *A. catechu*-treated group (Group B).

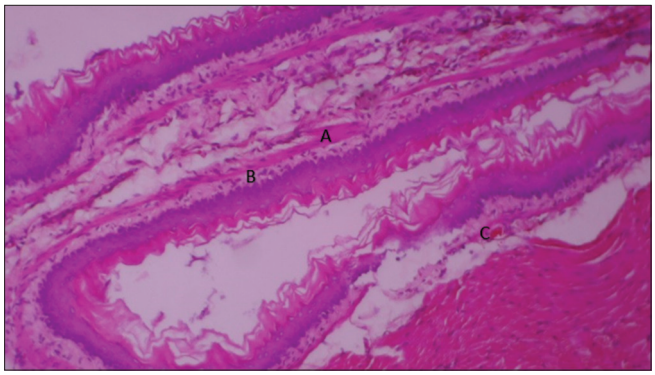

Figure 11: Rat esophagus given *Areca catechu* + 2000 mg/kg *Cymbopogon citratus* showing A: Normal epithelial lining, B: Mild subepithelial infiltrates of lymphocytes, and C: Mild active submucosal congestion (H and E, ×40)

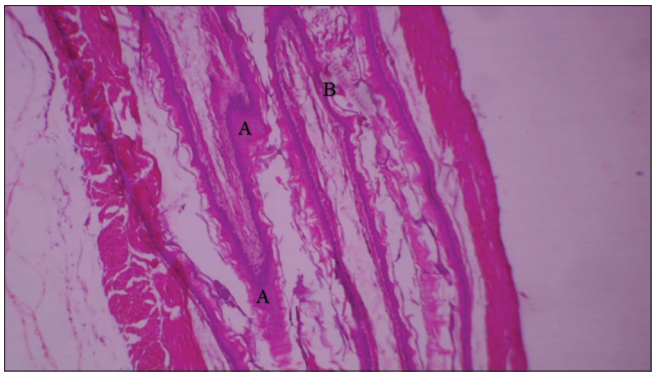

Figure 13: Rat esophagus given *Areca catechu* + 3000 mg/kg *Cymbopogon citratus* showing A: Focal thickening of the epithelium and B: Mild active submucosal congestion (H and E, ×40)

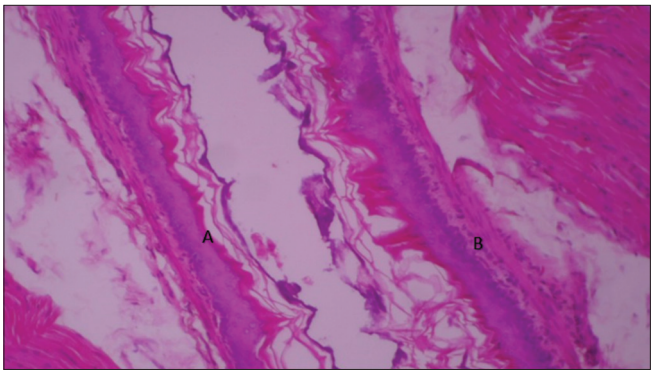

Figure 12: Higher magnification: A and B (H and E, ×100)

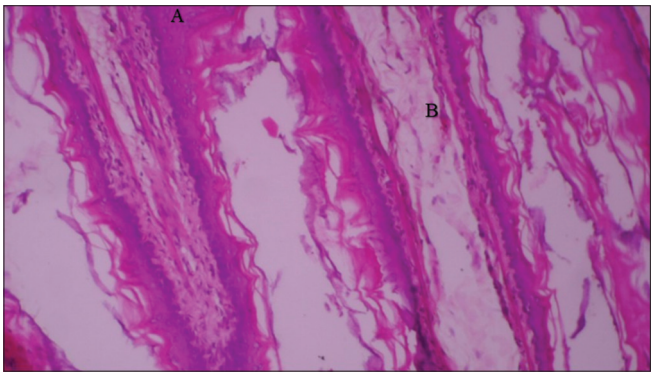

Figure 14: Higher magnification: A and B (H and E, ×100)

C. citratus was found to be a potent inhibitor of the esophageal irregularities, as the ingestion of test materials did not induce any proliferative lesions in the esophagi of *A. catechu*-treated Wistar rats. The rate of proliferation is the result of interaction between positive and negative regulators, which act via a complex control system involving the binding of peptide factors to cell surface receptors, a cascade of cytoplasmic elements regulated by the activities of kinases and phosphatases, and transcriptional activity in the nucleus leading to the expression of proteins involved in cell cycle regulation (Squier and Kremer, 2001). The microscopic observations of these papillomatous proliferations and lesions are harmonious with findings by Suri *et al*., who conducted a study on 65 male Syrian golden hamsters. The animals received topical applications on the cheek-pouch mucosa of areca nut extracts, tobacco, and areca nut plus tobacco weekly for 21 weeks, at which time all animals were killed. Local squamous cell carcinomas and leukoplakia were seen in 19/21 animals treated with areca nut extract. Dunham *et al*. (1968) also reported that application of arecoline to the cheek pouch of Syrian hamsters resulted in the induction of squamous cell carcinoma. Proliferation and atypia of basal cells in the esophageal papilloma were observed.

In Group B, the induction of local immunity was observed by the presence of lymphocytes. This phenomenon is in agreement with findings by Bhisey *et al.* who reported that areca nut interferes with the immune system by interfering with the activation of T cells and production of cytokines.

Researches have shown that *C. citratus* possesses high antioxidant capabilities (Pereira *et al*., 2009; Shah *et al*., 2011; and Koh *et al.*, 2012), and it is upon this premise that this research is based.

Conclusion

The researchers were able to induce esophageal papillary lesions by treating the esophagus of the Wistar rats with *A. catechu*. In addition, they were also able to combat *A. catechu*-induced esophageal lesions by the administration of *C. citratus.* The researchers, therefore, ascribe this phenomenon to the antioxidant and chemoprotective properties of *C. citratus,* as previous studies have shown (Devi *et al*., 2012).

We, therefore, recommend that consumption of *A. catechu* should be avoided, and that translational research be

carried out on *C. citratus*, so as to maximize its protective abilities on the esophagus.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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