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# MICROANATOMICAL EFFECTS OF ETHANOLIC EXTRACT OF *COLA NITIDA* ON THE STOMACH MUCOSA OF ADULT WISTAR RATS

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## Abstract

The study investigated the microanatomical effects of the extracts of *Cola nitida* on the stomach mucosa of adult male Wistar rats. Twenty adult male wistar rats were randomly divided into four equal groups of A, B, C and D (n=5). Animals in experimental groups B, C and D were given 600mg/kg body weight of crude extract of *Cola nitida* each by oral intubation for five, seven and nine consecutive days respectively, while group A (control) received equivalent volume of distilled water. Twenty four hrs after the last administration, the animals were sacrificed; tissues were harvested and fixed in 10% formol saline for histological analysis. The study revealed necrotized surface epithelium, degenerated gastric mucosa, and loss of glandular elements in the stomachs of experimental groups' vis-à-vis the control group. These observations were days-dependent; as those groups which received the extract for higher number of days were seen to be adversely affected. In conclusion, *Cola nitida* at 600mg/kg body weight can cause gastric lesion in animals. This lesion may be pronounced if the administration continued for days. *Cola nitida* should, therefore, be taken with caution to avoid gastric complications.

**Keyword:** *Cola nitida,* necrotized, degenerated, stomach mucosa, microanatomy, glandular elements.

## Introduction

Indigenous to Western Africa, the Cola tree (Kola tree) is cultivated today in many tropical climates, including Central and South America, the West Indies, Sri Lanka, and Malaysia. The vast majority of African Kola nut production is utilized within the African continent, particularly in sub-Saharan regions, which may explain why the introduction of the crop to the tropical Americas, the West Indies, Sri Lanka and Malaya has never caused severe competition with African production (Obika et al., 1996). The trees fruit annually when about twelve to fifteen years of age and may continue until they reach one hundred years, thriving in rich, well-drained soil in sun at a minimum of fifty-five degrees Fahrenheit. Among the vegetable products from the African soil, there is, perhaps, none more interesting and valuable than the African Kola nut, and it is consumed throughout tropical and equatorial Africa as an equivalent to tea, coffee, maté and cocoa. In Nigeria it is generally acknowledged that the Yorubas grow the kola nut; the Hausas eat it, while the Igbos celebrate it. It cuts across all the tribes in Nigeria; it is a symbol of hospitality. It is consumed by a large number of students, pregnant women, drivers and other menial workers as stimulant, masticatory, astringent and antioxidant. They are important in various social and religious customs (Russel, 1955;

Sundstrom, 1996; Haustein, 1971; Lovejoy, 1980). Of the various species of cola nuts, the two most commonly edible kinds are *Cola acuminata* and *Cola nitida*.

*Cola nitida* belongs to the genus *Cola* and family Sterculiaceae. They may be used to counteract hunger and thirst; used to control vomiting in pregnant women; used as a principal stimulant to keep awake and withstand fatigue by students, drivers, and other menial workers (Haustein, 1971; Chukwu et al., 2006). *Cola nitida* is not advised for individuals with stomach ulcers due both to its caffeine and its tannin content (Ibu et al., 1986, Newall et al., 1996). In traditional herbal medicine and folklore, *Cola nitida* was taken before a meal to improve digestion, and it has been reported that in Africa, its use exercised a favorable influence upon the digestive organs - particularly the liver - and most notably in the non-native populations who appeared to escape the constitutional changes due to affections of that organ; however, there are no clinical trials to prove this claim. *Cola nitida* is ingested daily by millions as one of the main ingredients in cola soft drinks. It is also used in diet and "high-energy" products such as food bars and as a flavoring in alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins, and puddings (Leung and Foster, 1996, Newall et al., 1996). Today, *Cola nitida* is still used as an alternative medicine mainly due to its antidepressant properties.

Investigations on the effect of *Cola nitida* on the stomach have not received much attention but it was reported to aggravate gastric and duodenal ulcer by increasing the level of gastric acid (Ibu et al., 1986). We therefore set to investigate the microanatomical implications associated with *Cola nitida* extract on the stomach mucosa.

## Materials and Methods

### Care and Management of Animals

A total of twenty adult male Wistar rats (220-250g) were used for this study; the animals were procured and maintained in the animal holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Nigeria. The animals were treated in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH, 1985).

### Extract Preparation

Kola nuts used for this study were fresh *Cola nitida* seeds (100g) procured in a local market in Ile-Ife, Nigeria. The nuts (voucher No.160A) were cut into pieces and dried; the dried seeds were ground into powder and was percolated with 70% ethanol and allowed for 3 days. Thereafter, the extract was filtered and the filtrate stored at -20$^{o}$ when not in use. The filtrate was freeze dried into a solid mass (94g). The extract was weighed (60g) and dissolved in 100ml of distilled water to give 600mg/ml of extract (Newall et al. 1996).

### Administration of Extract and Animal Sacrifice

Animals in group A served as the control and were administered with 1ml of distilled water equivalent to the volume of the extract administered to the experimental groups. Animals in groups B, C and D which served as the experimental group were given 600mg/kg body weight of ethanolic extract of *Cola nitida* via oral route using oropharyngeal tube for 5, 7 and 9 days respectively. Twenty four hours after the last administration, the animals were sacrificed by cervical dislocation; and following abdominal incision, the stomachs were excised and fixed in 10% formol saline.

### Histological Procedure

Histological study was carried out using the method of Carleton (1967)**.** These procedures involved dehydration of the stomachs with graded ethanol concentrations (50%, 70%, 90% and 100%, respectively), clearing in xylene, followed by infiltration in paraffin wax for 2 hrs at 56 $^{o}$C and embedding in paraffin wax for 48 hrs. Sections (5 μm thick) were then obtained, using a rotary microtome, subjected to Haematoxylin and eosin (H & E) staining procedure and examined under a light microscope. Permanent photomicrographs of the observations were taken, using an Olympus Research Microscope (model BX51).

## Results

Our microanatomical observations shed light on the mechanism of action by which *Cola nitida* extract cause gastric lesion under prolonged exposure. The results showed that the sections of the stomachs of the control were at variant with that of the treatment group. Sections from the control animals conformed to the basic histological features; the epithelium was found to be lined with simple columnar epithelium. The epithelium of the experimental animals was however found to be necrotized and degenerated with destruction of glandular elements (Figures 2-4). The mucosal damage was characterized by many apoptotic bodies just as the parietal and zymogenic cells presented a distorted arrangement in the gastric glands. The mucous neck cells were very scanty and displaced from their normal position when compared with the control group (Figure 1). The basic histological arrangements of the gastric layers were also seen to be irregular in the treatment groups vis-à-vis the control. These observations were observed to be days dependent as the gastric lesion were seen to be pronounced in groups that received the extract for several days i.e. groups C and D.

## Discussion

Our investigation has shown that administration of *Cola nitida* extract at 600mg/kg body weight is capable of causing gastric lesion in adult male Wistar rats. Several researches have been done on the effect of *Cola nitida* on the stomach and other parts of the gastrointestinal tract (Ibu et al., 1986; Blumenthal, 1998; Newall et al., 1996; Adegoke et al., 1998; Braid, 1990; Braid, 1991; Hussain et al., 1982; Okonji and Iwu, 1991; Pifferi, 1992; Uchida et al., 1992); these investigations were however deficient in that the microanatomical implications were neglected hence the need for this present study. The microanatomical implications of some of the hypothesis earlier acknowledged were investigated. This did not only provide us with relevant information about *Cola nitida*, but also added to our knowledge on the mechanism of action of this highly esteemed plant on the gastric mucosa. Sections of the stomach from the control group (Figure 1) were better organized in outline with intact glandular elements when compared with the experimental groups. Necrotized surface epithelium, degeneration of the gastric mucosa and destruction of glandular elements (Figures 2-4) observed in the experimental groups may be due to the high content of N-nitroso compounds found in *Cola nitida*. This may also be responsible for the aggravation of stomach ulcer earlier reported (Ibu et al., 1986; Newall et al., 1996). For instance, Ibu et al., (1986) discouraged the use of *Cola nitida* by ulcer patient due to its caffeine and its tannin content. Tannins, found in many plants, are substances that can irritate the stomach (Blumenthal, 1998).

The observed gastric lesion was believed to be pronounced due to continued administration of the extract for several days as those administered for few days only showed mild gastric distortion (Figure 2). These affirmed earlier report that *Cola nitida* should be used with caution and should not be taken over long period (Ibu et al., 1986). Other constituents of *Cola nitida* include theobromine, d-catechin, l-epicatechin, kolatin, kolanin, glucose, starch, fatty matter, tannins, anthocyanin pigment, betaine and protein (Newall et al., 1996). *Cola nitida* may not be toxic if mildely used (Blumenthal, 1998); reports however showed that in Nigeria, where the chewing of kola nuts is very common, there is a high occurrence of oral and gastrointestinal cancers, which may be due to this practice. The destruction of the glandular elements (characterized by many apoptotic bodies) in our present study was concomitant with the destruction of the cytoplasm of the parietal and zymogenic cells thus leading to the discharge of their contents into the gastric lumen thereby increasing the gastric acid content. This may be one of the mechanism by which *Cola nitida* brings about the ulceration of the gastric mucosa observed in this present investigation as well as previous works. These observations conform to earlier reports that kola nuts have fat-burning properties and stimulate the secretion of gastric juices (Esimone et al., 2007; Ibu et al., 1986). The gastric juices, so indiscriminately discharged, are capable of digesting the surface epithelium if not put under control (Ibu et al., 1986). *Cola nitida* is widely consumed by quite majority and still in use as an alternative medicine; this may be mainly due to its caffeine content and antidepressant properties (Ibu et al., 1986; Newall et al., 1996; Esimone et al., 2007). *Cola nitida* has been used in folk medicine as an aphrodisiac and an appetite suppressant, and to treat morning sickness, migraine headache, and indigestion (Esimone et al., 2007). It has also been applied directly to the skin to treat wounds and inflammation (Newall et al., 1996). The tree's bitter twig has been used as well, to clean the teeth and gums (Esimone et al., 2007). In addition, the tonic effect stimulates and tones up the nervous system and imparts an overall feeling of healthy well-being. It has been used to alleviate nervous debility, temporary depression, despondency, weakness, nervous diarrhea, anxiety and lack of emotion.

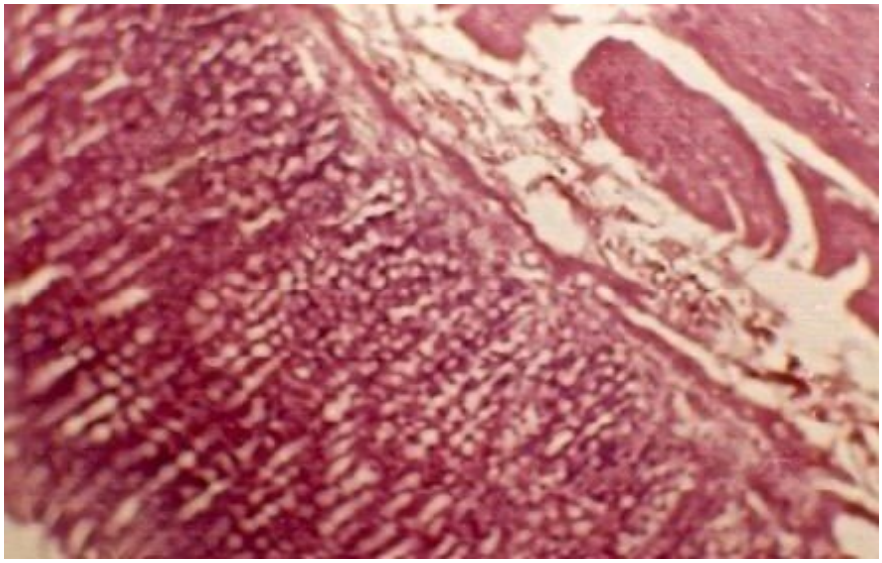

**Figure 1:** Section of the control stomach showing the four layers in the gastrointestinal plan. Note the well organized and preserved mucosa as compared with Fig. 2-4. H & E x100

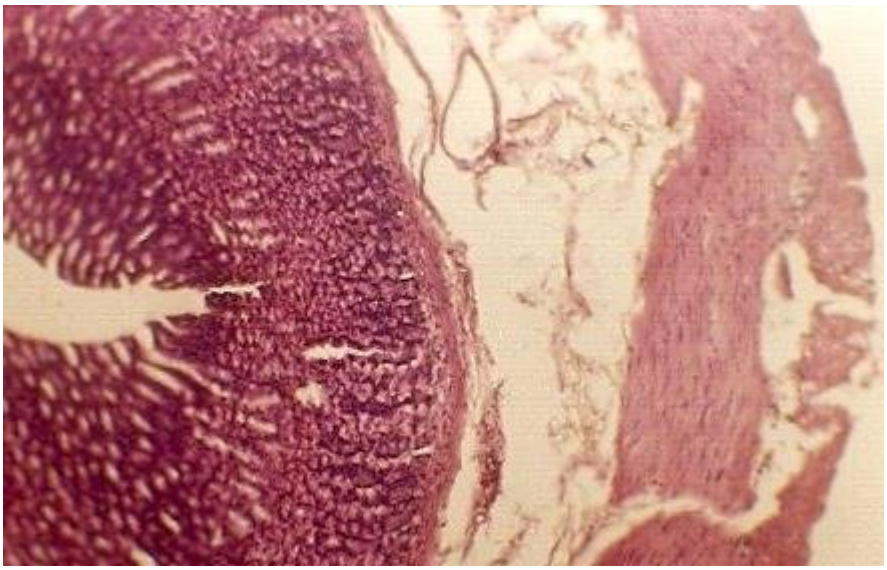

**Figure 2:** Section of treated stomach showing gradually eroded epithelium H & E x 100

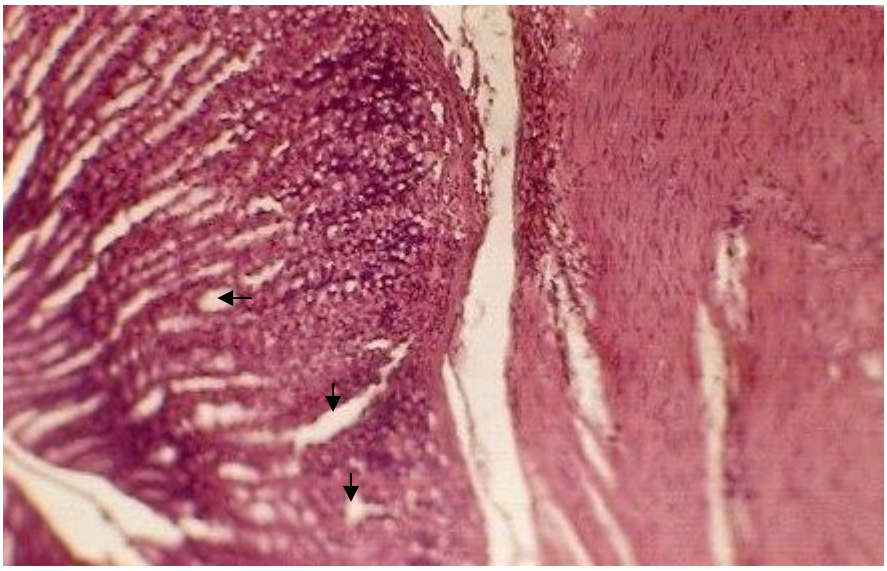

**Figure 3:** Section of treated stomach showing eroded epithelium. Note the vacuolations in the mucosa (arrow) H & E x100

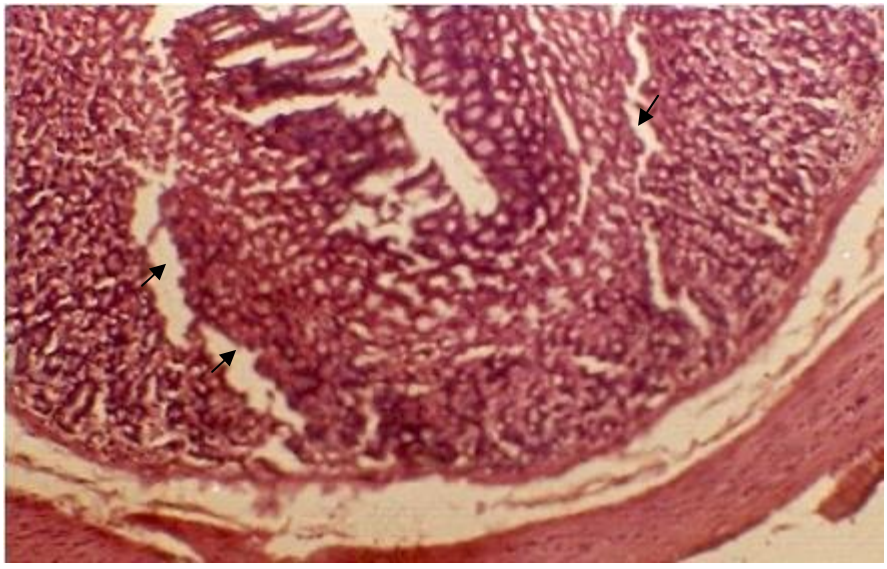

**Figure 4:** Section of treated stomach showing eroded, necrotized epithelium and vacuolated mucosa (arrow) H & E x100

In conclusion, *Cola nitida* may have so many beneficial properties among which have been mentioned above, indiscriminate or prolong usage may lead to necrotized surface epithelium, degeneration of the gastric mucosa and destruction of glandular elements as seen in this investigation. These are detrimental to the health of an individual and may lead to gastric complication. We therefore advocate the proper usage as recommended by Germany's Commission E (Blumenthal, 1998).

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