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Histological effect of chronic ingestion of *Garcinia kola* seed on the pancreatic acini of adult wistar rats.

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Abstract

Garcinia kola seed is a widely consumed plant product. This study was undertaken to assess the effect of its consumption on the pancreas, an important digestive gland. Adult male wistar rats weighing 150-200g, (7-10 weeks) old were used for the study. They were divided into a control and three experimental groups. The control group received the normal feeds only for the same period while the experimental groups were fed dry powdered seeds of *Garcinia kola* (GK) mixed with the rat feeds at a ratio of 5%w/w (200g/400g of feeds), 10%w/w (400g/400g of feeds) and 15%w/w (600g/400g of feeds) for six weeks. At the end of the experiment, animals were sacrificed by ether anesthesia and the pancreas was dissected out and preserved in 10% buffered formal saline. It was processed using routine histological procedures and stained with haematoxylin and eosin (H&E) stains. Results obtained indicated that GK seed caused cellular changes such as vacuolation of the epithelial cells of the pancreatic acini. The group which received the highest dose 200g of GK powder per 400g of feeds mixed together was affected more with pale secretory cells, and pyknotic nuclei. These changes may be attributed to one or more of the constituents of GK seed such as benzophenone derivative which has cytotoxic activity.

Key Words: *Garcinia kola,* chronic ingestion, pancreatic acini, rat, histology.

Introduction

Plant products as sources of medicinal compounds have made significant contributions to human health and well being (1). Traditional medicine also referred to as complementary alternative medicine, is widely gaining grounds and acceptability in Nigeria. Natural products play important role in drug development programs of the pharmaceutical industry (2,3). In developing countries, especially in rural context, people usually turn to traditional medicine when ill and plants of ethnobotanical origin are often used (4). In the western world (USA), it is estimated that 18% of adults use prescription drugs concurrently with herbal or vitamin products (5). Different species of plants have been recognized to occur in the African continent and many of them have been found to be very useful in traditional medicine (6,7). *Garcinia kola* (Gk) nut is one of these plant products used.

Gk nut is the seed of a tropical fruit obtained from a large forest tree belonging to the family, Guttiferrae. The Igbos call it *Akpi-ilu*, the Yorubas call it '*Orogbo*' the Efiks call it '*Efiari*' while the Hausas call it '*Namijin goro*'. This seed is commonly consumed for pleasure and as a therapy for some ailments such as cough, colic (8), bronchitis, inflammation of the respiratory tract and liver cirrhosis (9). It has antimicrobial property (10,11,12,13), anti inflammatory action (14) antioxidant effect (15,16), antihepatotoxic (17,18,19,20). It is also used as an aphrodisac (21). It has antimalarial (22) and antidiabetic (23,24) effects. The seed is composed of unsaturated fatty acid particularly oleic and linoleic acid, (25). The major component of the seed is a biflavonoid called '*kolaviron*'. Flavonoids are polyphenolic substances which have recently aroused interest as a result of the biological

activities such as antimicrobial, antioxidant and antimutagenic activities attributed to it (26). Despite the wide application of this seed in Traditional Medicine, Gk seed has been reported to destroy the cells of the proximal convoluted tubules of the kidney, liver hepatocytes and villous epithelium of the intestine after feeding rats with a diet of dry powdered seeds of GK for six weeks (27). It caused necrosis of the germinal epithelium in the testis of rabbits fed with 35mg/kg (28).

The pancreas is a large lobulated compound tubuloacinar gland. The exocrine portion is a branched acinar gland surrounded by delicate connective tissue. The cells are arranged round a central lumen. The basal cytoplasm is basophilic while the apical cytoplasm contains acidophil secretive (zymogenic) droplets or granules, the endocrine portion are scattered throughout as spheroidal mass of pale staining cells. The histological and morphological features of the pancreas may be altered due to some diseased conditions or ingestion of toxic substances. The aim of this study was to find out the effect of chronic consumption of *Gk* seed on the acinar cells (exocrine portion) of the pancrease.

Materials and method

Plant Material.
The seeds were obtained from a market in Akpabuyo local Government area of Cross River State. The outer coats were peeled off, cut into pieces and air- dried. It was grounded into powder and different quantities were mixed with the rat feeds at different ratios and fed to the rats.

Animal treatment:
The animals were obtained from the animal house of the Physiology Deptpartment, University of Calabar. They were kept under standard room temperature of 27-29^{0C} and 12 hours light: 12 hours dark cycles. They were fed with rat feeds obtained from Gems Verterinary and Pharmaceutical Services Ltd, Atu street, Calabar. They had access to drinking water *ad libitum*. They were divided into four groups consisting of five rats each weighing about 150-200g. The groups were as follows:

Group A - Control group, received normal feeds only.
Group B - received 5g of Gk seed powder per 100g of feeds, (5%w/w)
Group C - received 10g of Gk seed powder per 100g of feeds, (10%w/w)
Group D - received 15g of Gk seed powder per 100g of feeds, (15%w/w)

All treatments lasted for six weeks at the end of which the animals were sacrificed by chloroform anesthesia. The pancreas was dissected out and preserved in 10% buffered formalin. The organ was trimmed and processed histologically for H& E staining.

Results:
Histological sections of pancreas from control group showed secretory acini cells with their darkly stained nuclei located at the base of the cells. The outline of the acini cells were distinct. The endocrine portion were seen as isolated patches of cells interspersed among the secretory acini (Plate 1). In the group which received 5%w/w of Gk seed powder, section appeared quite similar to those of control group. The acini cells were distinct and nuclei

prominent (Plate 2). Histological sections from animals which received 10%w/w of Gk powder showed pale secretory acini, with areas of vacuolations of the cells. The cell outlines were not distinct as in control group and nuclei were not prominent. (Plate 3).

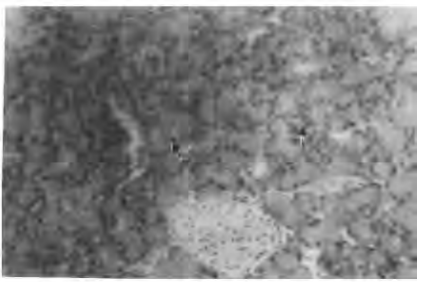
In the group which received 15% w/w dry powdered seed of Gk, the pancreatic acini cells outlines were not distinct. A decreased uptake of the stains and vacuolation of the secretory acini were observed. (Plate 4).

PHOTOMICROGRAPHS.

Plate1:

Section of the pancreas of rat showing normal spheroidal pancreatic acini surrounded by connective tissue. Interspersed among the acini are the Islet cell. Mag. x100 (H & E).

Plate 2:



Section of pancreae from rat treated with 5% (w/w) of *Garcinia kola* seed powder for 6 weeks showing no adverse effects. Sections are similar to control sections. Mag. x100. (H & E stains).

Plate 3:

Section of pancreas from rat treated with 10% (w/w) of dry powdered seed of *Garcinia kola* for 6 weeks showing pale staining secretory acinar cells and some areas of vacuolations (arrow). Mag. x100. (H&E stains).

Plate 4:

Section of pancreas from rat treated with 15% (w/w) of dry powdered seed of *Garcinia kola* for 6 weeks showing indistinct cell outline and vacuolations of the secretory Acini (Arrow heads). Mag. x100. (H & E stains).

Discussion

Gk seed was observed to be toxic to the excocrine portion of the pancreas. This may be attributed to the presence of some constituents such as tannins and benzophenones which

have been reported to have cytotoxic activities (27,29). Consumption of low dose of Gk seed powder did not present any observable adverse effect histologically but high dose caused vacuolation of the secretory acini. The exocrine portion of the pancreas produces pancreatic juice which contains enzymes for completion of digestion of protein and fat. It has been reported that Benzophenone derivatives which is one of the constituents of *Gk* seed, which is the basis for the antimicrobial activity of Gk seed is relatively a potent inducer of the Phenobarbital type of 2-3 Cytochrome P_{450} enzymes (liver microsomal cytochrome P_{450} 2B isomer), (29). This enzyme may be responsible for metabolizing the Gk seed powder into a toxic metabolite capable of destroying the cells.

The toxic effect of Gk seed reported on the liver hepatocytes, proximal tubule of kidney and intestinal villous epithelium by (27) was attributed to the presence of tannins as one of the constituents of Gk seed. These organs in which the toxic effect of Gk has been reported are made of epithelial cells, which seems to be the target organ of toxicity. Epithelia are membranes composed of cells with basement membranes (30). They serve as selective barriers for the entry of substances to organs which they line. These membranes contain lipid layers through which free radicals can attack leading to oxidative damage of the organ (30). Gk seed contains unsaturated fatty acid and the oxidation of polyunsaturated fatty acid in cells leads to toxicity, lipid peroxidation and cell damage (30). However, Gk seed and its biflavonoid has been reported to inhibit lipid peroxidation (31,17,32). In the present study, the adverse effect observed in the groups which received 10%w/w and 15% w/w concentration consumed (groups C and D animals) may have been toxic due to the presence of other constituents and not the flavonoids. The result obtained from this study suggests that while low dose of Gk seed may not be toxic, chronic/high dose consumption may have toxic effects. Gk seed may have a dose dependent toxic effect on the cells of pancreatic acini.

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