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Gastric mucosal changes in indomethacin-induced ulcers following long term oral feeding with coconut extracts in male rats.

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ABSTRACT

Changes in the histology of mucosa with induced gastric ulcers following oral feeding of coconut milk and water in male albino rats were studied. 40 male albino rats divided into four study groups made of ten animals were used for the study-control (group i), coconut water fed (group ii), coconut milk fed (group iii) and sucralfate fed (group iv). Aqueous test substances were administered by gavage daily after ulcer induction for 28 days. Gastric mucosal ulcerations were induced with indomethacin administered subcutaneously. The animals were allowed free access to food and water ad libitum. Stomach specimens were subjected to histopathological anlaysis. The results of the analysis showed that there were extensive gastric pits and ulcer sites with mononuclear (leucocyte) cellular infiltrations with loss of gastric glands and chronic inflammation were observed. The photomicrographs of the coconut milk and sucralfate groups showed similar cytostructure with reduction in the ulcer diameters, pin-point ulcers, more abundant mononuclear cellular infiltrations. These findings reflect cytoprotection and healing of the gastric mucosal structure damaged by indomethacin-induced ulceration. That the same effects were observed in the coconut milk and sucralfate groups but not in the coconut water confirmed the conventional usage of sucralfate as a cytoprotective agent. It is concluded that coconut milk provided a stronger cytoprotection than coconut water, and that coconut milk may have acted via same pathway as sucralfate.

Keyword; coconut, ulcerations, cytoprotection.

Folkloric medicine in Nigeria employed coconut water dispersion and fleshy endocarp (meat) as antidote for poisoning, over dosage of drugs and untoward drug reaction (Osazuwa and Ahonkai, 1990). Coconut, generic name is cocos nucifera (palmitae). Coconut water is the aqueous dispersion found in coconuts. It is sterile provided it is fresh until exposed to micro-organisms (Eisman. 1954; Osazuwa and Ahonkai, 1990). Coconut milk and dispersion contain amino acids like glutamic acid, arginine, leucine, lysine, proline, aspartic acid, tyrosine, alanine, histidine, phenyalanine, serine and cysteine (Pradera et al, 1942). Coconut is rich also in fatty acids and oils with the largest constituents as lauric, myrstic, palmitic, capric, stearic and unsaturated ones such as oleic and linoleic acids (Adodo, 2002). Coconut water is highly nutritive and its usage as a natural ORT has been suggested (Kuberski et al. 1979; Ogonor 1988). Animal models showed that animals given aloe vera gel after developing ulcers recovered three times faster than the control animals (Galal et al, 1975). It has been shown to prevent stomach lesions pretreatment by 85 percent and 50 per cent better than the controls in healing gastric ulcerations (Kandil and Gobran, 1979). Exposure of gastric mucosa to ulcerogenic, and or necrotizing and ischaemic agent such as aspirin, indomethacin, bile acids, alcohol, ether and steroids cause gastric damages (Jones et al, 1999). This present study investigated the effects of coconut milk and water in indomethacin-induced gastric ulcers in male rats.

MATERIALS AND METHODS

Animals

40 male albino rats weighing 190-250g raised in the College Animal House were randomly selected and divided into four groups of ten rats (groups I, II, III, and IV). They were kept in adequate conditions and fed with normal rat chow (Pfizer Feeds, Aba, Nigeria), had free access to water ad libitum. The ethical standards by the Ethical Committee of the College of Medicine and Health Sciences were observed.

Induction of gastric Mucosal Ulcers

Gastric mucosal ulcers were induced using indocid in 25mg.5ml suspension (indomethacin) (MSD, Canada) administered subcutaneously at a dose of 40mg/kg body weight in all animals according to the methods described by Njar et al. (1994, 1995). The rats were fasted 12-18 hours prior to ulcer induction and they had free access to water.

Solution and extract preparation

Sucralfate was prepared by dissolving it in 100ml of distilled water and was administered orally.

Coconut milk extraction

The native coconuts used were purchased from Choba market, Port Harcourt. The coconuts were cracked and the coconut water was collected in a beaker, and measured. The fleshy endocarp (white meat) was removed, placed in blender (Nakai blender, 1906) for grinding it, and 200ml of warm distilled water was added then coconut milk was collected via filtering through clean cheese cloth. The crude coconut milk extract and the aqueous dispersion of coconut water served to the rats with ulcers.

Feeding experiments

The animals in groups II, III, IV were given the test substances by gavage daily starting from four hours post ulcer induction. Group I received oral distilled water (2.0ml daily), group II received aqueous dispersion of coconut water (2.0ml daily), group III warm water extract of coconut milk (2.0ml daily) and group IV received sucralfate, basic aluminium sulphate sucrose (14.28mg/kg body weight) given at 4.76mg/kg body weight three times a day determined from prescribed dosage for humans (Brunt et al. 1988). All the animals in the different groups were given free access to food and water ad libitum. The test substances were administered for twenty-eight days.

Histochemical analysis

After 28 days, the animals were sacrificed via cervical fracture. A midline laparatomy was done to remove the stomach. Then, the stomach was cut open along the greater curvature from the oesophageal to the site of duodenal fistula end. The stomach was rinsed in 0.9% saline to remove any debris. The stomachs were stored in 10% formol saline prior to tissue and histological processing. Sliced portions of the stomach specimens were subjected to various stages of tissue processing starting from preservation to dehydration using increasing level of alcohol. The tissues were cleared in xylene and infiltration was done in molten wax before they were embedded on clean paraffin wax. After embedment, the distance of 5-7 microns in a microtome, then they were stained routinely using haematoxylin and eosin dyes. The stained slices were mounted on plain slides and photographed with a close micro lens camera and the photomicrographs are presented as figures at X400 magnification. The morphological changes were evaluated by analyzing the photomicrographs.

RESULTS

Result are presented in photomicrographs. Histological sections revealed extensive excoriations, gastric pits and ulcer sites with infiltrations of mononuclear cells and eroded gastric mucosa in the control (Fig 1). Loss of gastric glands and chronic inflammation with increased mononuclear cellular infiltration (MNCI) as well as eroded areas of mucosa less than 1mm and gastric were observed in the group II (Fig 2). Pin-point ulcers with abundance of large mononuclear cellular infiltrations (MNCIs) and reduction in size of ulcer sites, eroded mucosa with diameter less than 1mm (Figs1 & 2).

DISCUSSION

The present study investigated the histological changes in indomethacin-induced gastric mucosal ulcerations following long term administration of coconut water, coconut milk and sucralfate. The visible and extensive gastric pits and ulcer sites with mononuclear cellular infiltrations, eroded mucosa and gastritis observed in (Fig. 1) was consistent with erosions of gastric mucosa and gastritis reported in the rats which had ulcers (Lambert, 1968).

It suggests that the ulcerogenic agent, indomethacin used in this study could have interfered with ulcer healing process by inhibiting epithelial cell proliferation, migration and

Figure 1: photomicrograph of gastric mucosa in the control group after 4 weeks showing extensive gastric pits and ulcer sites with eroded gastric mucosa and infiltration by mononuclear cells. Stained preparation, H & E, X400.

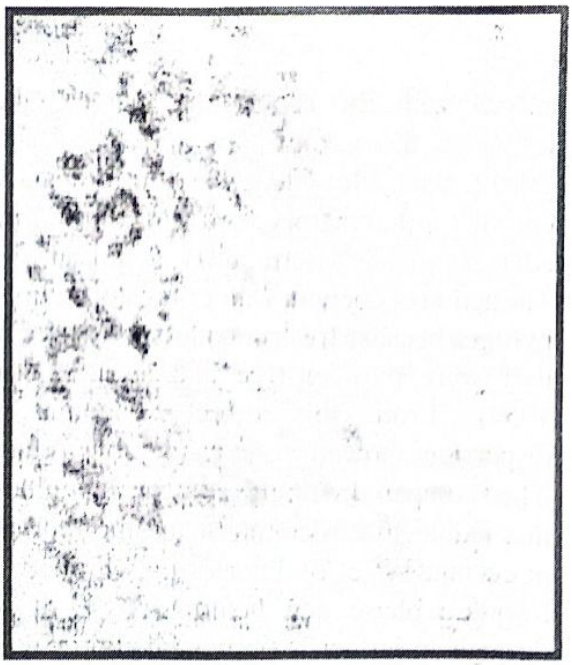

Figure 2: photomicrograph of gastric mucosa in the coconut water fed rats after 4 weeks showing loss of gastric glands, gastritis, chronic inflammation increase mononuclear cellular infiltration, as well as eroded of the mucosa of diameter < 1mm . Stained preparation, H & E, X400.

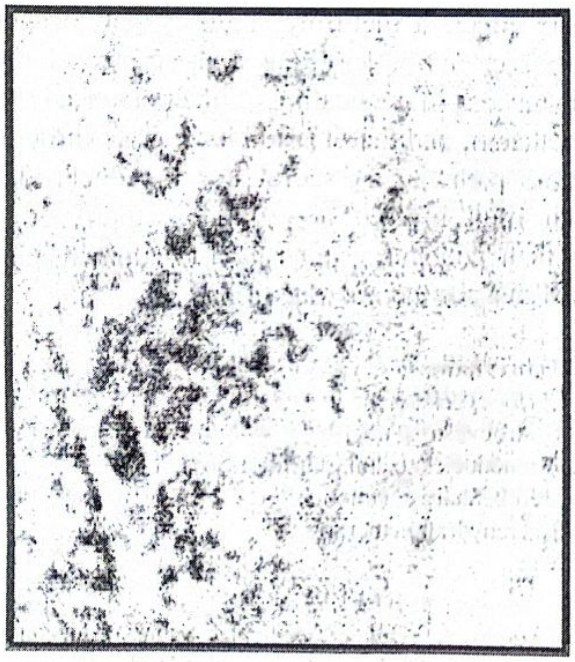

Figure 3: Photomicrograph of gastric mucosa in the coconut milk fed rats after 4 weeks showing more intensive gastritis, pin point ulcers/punctuate haemorrhages, large and abundant mononuclear cellular infiltrations with eroded mucosa in diameter by 1mm. Stained preparation, H & E, X400.

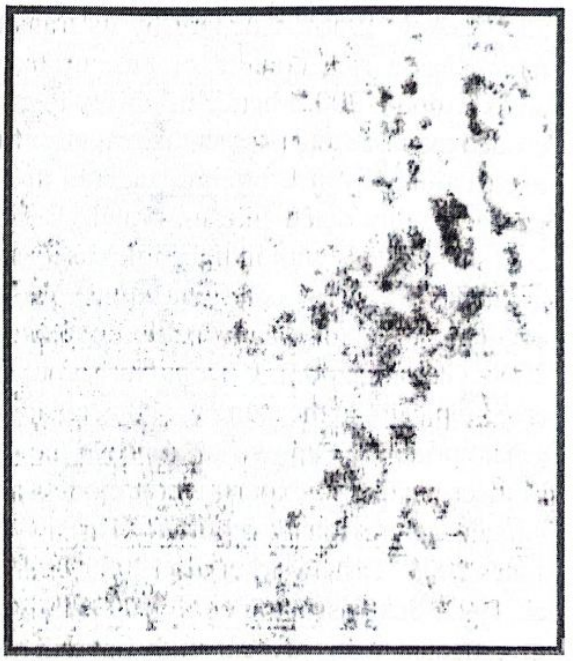

Figure 4: photomicrograph of gastric mucosa in the sucralfate fed rats after 4 weeks showing pin-point ulcers < 1mm in diameter with abundance of mononuclear cellular infiltrations. Stained preparation, H & E, X400.

angiogenesis, and by blocking growth factor-triggered signaling pathways (Jones, et al, 1999a, b; Pai et al, 1998; Tarnawaski and Jones, 2005). The increase in mononuclear cellular inflammations (MNCIs) with the loss of gastric glands, presence of chronic inflammation, eroded mucosae less than 1mm in diameter observed in the coconut water group (fig. 3)

agreed with the report that some unhealed lesions at three, four, six weeks also showed granulomata with ulcer base accompanied by chronic inflammatory cell infiltration in the adjacent glands (Bertalanffy and Lau, 1962). The action of coconut water may not be linked to pyrogen because fresh coconuts were used hence they were pyrogen-free and sterile (Eisman, 1954). From this effect, coconut water dispersion showed a considerable protection when compared with the control. It could imply that indomethacin counteracted the mild action of coconut water by interfering with both acute response phase and healing phase of repair through an influence on wound contraction and and mucosal regeneration (Townsend, 1961). The activities of coconut milk and sucralfate on ulcerated gastric mucosa suggest both protection as shown by increased MNCIs, reduction in diameter of eroded mucosae and pin-point ulcer sizes via enhancing increased mitosis (Leblund and Walker, 1956). Coconut by its translucent nature has a rich content of unsaturated fatty acids (Adodo, 2002) hence its ability to coat the eroded mucosae and protect them against further excoriation by necrotizing agents and high gastric acidity acted like milk and cheese that coat the mucosa with indigestible casein as well as delay gastric emptying thus providing adequate time for neutralization (Rangwani, 2006: Ganong, 2003). Coconut milk could have acted topically in the same way that antacids and rebamipride are known to improve the quality of ulcer healing in experimental models and in humans as previously reported (Tarnawski and Jones 2003: Tarnawski et al, 1998; Arakawa et al. 1993; Schmassmann et al, 1993) though not investigated in this present study but will be done in future. When the action of coconut milk was compared with that of sucralfate, an aluminium containing cysprotective agent, they were similar (fig. 3 & 4). Thus the abundant increase in MNCIs in coconut milk and Sucralfate groups but not in the coconut water group showed that both coconut milk sucralfateand milk and sucralfate were effective against indomethacin-induced ulcers in rats. As a result, the comparable and similar actions of coconut milk and sucralfate confirmed their cytprotection of gastric mucosal wounds. Ulcer formation induced by indomethacin is known to be related with inhibition of cyclooxygenase (COX) that prevents prostaglanddins biosynthesis; which in turn inhibits the release of mucus, a defensive factor against gastrointestinal tract (GIT) damage (Bandyopadhyay et al. 2000). Ding et al (1998) showed that recruitment and activation of mono nuclear cellular (Leucocytes) infiltration in gastric mucosa are crucial in indomethacin-induced gastric injury as mucosal damage peaked at six hour post ulcer induction by indomethacin hence we commenced feeding four hours after inducing ulcers. Olive oil contains oleic acid like coconut milk. Ozbakis Dengiz and Gursan (2005) showed that olive oil extract of Mormodica charatia L. decreased polymononuclear leucocyte infiltration in indomethacin-induced ulcer in a rat model and thus their result was not consistent with our observations with coconut milk which caused an opposite effect. It therefore suggests that coconut milk extract in this present study protected the gastric mucosa in the healing of ulcers induced by indomethacin, and that it could have acted through the same pathway as sucralfate. In conclusion, coconut milk offered a stronger cytoprotective effect than coconut water against indomethacin-induced gastric mucosal ulcerations.

List of Abbreviations:
COX - Cyclooxygenase
GIT - Gastrointestinal tract
MNCI - Mononuclear cellular infiltration
MSD - Merck, Sharp & Dohme
ORT - Oral rehydration therapy

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