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Evaluation of gastroprotective potentials of papain and fucoidan in wistar rats

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Abstract

Background: Gastric cytoprotection involves acid-independent protection of gastric and intestinal mucosal cells against injurious agents. Papain is a proteolytically active enzyme derived from *Carica papaya*, while fucoidan is a sulfated polysaccharide rich in fucose and sulfate obtained from brown seaweeds.
Objectives: This study assessed the comparative effects of papain, fucoidan and metoclopramide on cytoprotection in Wistar rats.
Method: Twenty male Wistar rats (100-150g) were divided into four groups (n=5). Group 1 served as the control group (Ctrl group), which was administered water and rat feed. Group 2 served as the Papain group (Pap group) and was administered papain at a dose of 800 mg/kg. In comparison, group 3 served as the Fucoidan group (Fuc group) and was administered Fucoidan at a dose of 800 mg/kg, and group 4 was the Metoclopramide group (Met group) and received Metoclopramide at a dose of 30 mg/kg. All administrations were given orally, once daily, for twenty-eight days.
Result: Pepsin output showed no significant difference across groups. Mucus secretion and ulcer score increased significantly in all test groups compared with the control. Mucus secretion was higher in the Met group than in the Pap group. The ulcer score was lower in the Met group than in the Pap group. Histology showed distorted duodenal architecture in the Pap and Met groups, with preserved structure in the Fuc and control groups.
Conclusion: Fucoidan is recommended as a promising candidate for the management of gastric and intestinal mucosal disease disorders, while caution is advised regarding the prolonged use of papain.

Key words: Gastric cytoprotection, Gastroprotection, ulcer-score, mucus secretion, papain, fucoidan and metoclopramide.

Introduction

Cytoprotection refers to the process by which certain pharmacological agents protect cells, particularly the gastric and intestinal mucosal cells, from injury caused by harmful substances, without inhibiting or neutralising gastric acid.[1] A gastric cytoprotectant is any medication that combats ulcers not by reducing gastric acid but by increased mucus and bicarbonate secretion, enhancement of mucosal blood flow, cellular repair, and antioxidant activity.[2] Cytoprotectants can be categorised into enzyme and polysaccharide-based.[3,4]

Papain is a purified, dried enzyme preparation obtained from the latex of unripe fruits and leaves of

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Carica papaya L., a member of the family Caricaceae.[5]

Papain is the English translation of 'papaïne', the name given by Wurtz and Bouchut[6] to a proteolytically active constituent in the latex of the tropical papaya fruit (*Carica papaya*). Papain, a proteolytic enzyme found in papaya, contains several key phytochemical constituents. These include the enzyme papain itself, as well as chymopapain, pseudocarpain, and lysozyme.[5] Additionally, papaya, from which papain is extracted, contains other phytochemicals like carotenoids (β-carotene and cryptoxanthin), flavonoids, and various minerals[7] Papain is the most well-known, but the latex of *Carica papaya* also contains enzymes such as chymopapain, caricain, and protease omega, flavonoids, carotenoids minerals like calcium, iron, magnesium, potassium, zinc, and manganese.[8] Other Phytochemicals present in various parts of *Carica papaya* include: tannins, saponins, alkaloids, and glycosides, particularly in the leaves; amino acids, and organic acids such as citric and malic acids.[9,10]

Papain contains 15.5% nitrogen and 1.2% sulphur. Crystalline papain is most stable in the pH range 5–7 and is rapidly destroyed at 30°C, below pH 2.5 and above pH 12.8 Papain is a protein of 212 amino acids and has a molecular weight of about 23,000 daltons. It is resistant to heat, inactivated by metal ions, oxidants and reagents which react with thiols, and is an endopeptidase activated by thiols and reducing moieties (cysteine, thiosulphate, and glutathione).[11]

Seaweeds are a group of photosynthetic species that produce several valuable compounds as metabolites with multiple uses. Most algae are mainly made up of polysaccharides on a dry weight basis. They exhibit bioactive properties and are applicable as ingredients in functional foods and nutraceuticals.[12]

Fucoidan is a sulfated polysaccharide found in brown seaweeds and some marine invertebrates and primarily consists of fucose and sulfate.[13]

Fucoidan is a non-starchy form of carbohydrate since its sugar units are not linked by either α-1-4 or α-1-6 glycosidic bonds.[14] It is because of their non-starchy nature that fucoidan is not hydrolysed by digestive enzymes; however, the probiotic microbes in the colon of humans could ferment this polysaccharide. Fucoidan is a polymer of α-1–3 linked fucose pyranose sugar subunits and has traces of galactose, xylose, and glucuronic acid.[15] It is composed of 44.1% fucose and 26.3% sulfate.[16]

Fucoidan, a sulfated polysaccharide containing mainly l-fucose and sulfate,[17] has been recognized as a multifunctional substance with many physiological and biological activities,[16] including anti-inflammatory, anticoagulant, antithrombotic,[18] antiviral, immunomodulatory, antioxidant, and antitumor agents.[19]

Materials and methods

Experimental protocol

Twenty male Wistar rats (100-150g) were used for the study; they were divided into four groups of five rats each. Group 1 was the Control group (Ctrl group) and was allowed free access to water and rat feed. Group 2 served as the Papain group (Pap group) and was administered papain at a dose of 800 mg/kg body weight, while group 3 served as the Fucoidan group (Fuc group) and was administered Fucoidan at a dose of 800 mg/kg body weight. Group 4 was the Metoclopramide group (Met group) and served as the positive control, which received Metoclopramide at a dose of 30 mg/kg body weight. All administrations were done orally and once daily for twenty-eight (28) days.

Collection of Pepsin

Gastric juice used for analysis of pepsin was collected according to the method of Shay et al. 1964 as used by Zullo et al.[20] The animals were fasted for 36 hours to ensure that their stomachs were completely empty. Water was added ad libitum, under light chloroform anesthesia, the abdomen of each rat was shaved, and midline incision was made extending 2cm downward from the xyphoid, the junction between the pylorus and the duodenum was picked gently with a curved probe. The stomach itself was not disturbed; a pyloric ligature was made using silk thread and applied carefully to avoid damage to the blood or traction on the stomach. The abdomen was then closed by interrupted sutures. The abdominal walls were cleaned thoroughly with physiological saline, dried and covered with a solution of flexible collodion. The anaesthesia was discontinued, and the animal usually regained consciousness within 10 minutes. Four hours later, the animals were again anaesthetised with chloroform, the abdomen was opened, the pylorus, duodenum and appropriate peritoneal ligature were clamped. The stomach was removed, rinsed in physiological saline, and dried. An opening was made along the greater curvature and the gastric juice was drained into a small centrifuge tube and then centrifuged at 3000rpm for 10 minutes and the

supernatant was collected for pepsin analysis.

Measurement of Pepsin

The determination of proteolytic activity of gastric secretion (which is the basis for measuring pepsin) was performed using casein as a substrate according to the method of Zullo et al.[20] One ml of various concentrations of bovine casein ranging from 0.1-1.0mg/100ml in 0.1N HCI was transferred to a tube and incubated for 30 minutes with 3.9 ml of 259/100 ml 0:1.N HCI of the substrate in a water bath at 37° C. 10ml of 10% trichloroacetic acid (TCA) was added and the tubes allowed to stand for 10 minutes before they were filtered using Whatman's filter paper. Blanks were made for each concentration by adding 10ml TCA before the addition of the enzyme. Duplicate determinations were performed for each enzyme concentration. The optical density of the filtrate was measured at 280nm wavelength. For determination of the proteolytic activity of gastric secretion, the same procedure was followed at a concentration of 2% of 0.1N HCI (by adding 1ml of 2% of 0.1N HCI into each). A standard curve was constructed from which the pepsin content of gastric secretion was determined.

Determination of Mucus Secretion

The adherent gastric mucus was determined by the method described by Archibong et al.[21]

The animals were fasted overnight prior to commencement of the experiment, after which they were sacrificed and their stomachs removed. Each stomach was opened along the greater curvature and spread out on a board, and using pins to hold the edges, a spatula was used to scrape off the surface of the mucosa and introduced into a pre-weighed, sterilized sample bottle containing 3 ml of distilled water. The sample bottle was re-weighed after mucus collection on a sensitive electronic balance. Mucus output was then obtained as the difference in weight between the sample bottle containing water and the sample bottle containing water and mucus. Values were recorded.

Determination of ulcer score

Each animal's stomach was isolated, washed and cut open along the greater curvature and rinsed with normal saline. Pins were used to fasten the tissues in place for proper visualization. Magnifying lens and Vernier caliper were used to measure the extend of ulceration. Ulcer scoring was done by the method Alpin and Ward, 1967 as modified by Osim et al.[22]

Histological study

The harvested duodenum was cleared and fixed in Bouin's fluid and thereafter dehydrated with ethanol and embedded in blocks of paraffin. Tissue blocks were sectioned and stained with hematoxylin and eosin (H & E) and examined microscopically at x400 magnification.

Statistical analysis

All results were expressed as mean ± SEM, and comparisons across groups were performed using ANOVA. $P<0.05$ was considered significant. Graphpad Prism version 8.2 was used for the analysis.

Results

Comparison of pepsin output across control group and test groups

The mean values of pepsin output were 0.450±0.0100, 0.470±0.0100, 0.480±0.0200 and 0.475±0.00500 for control, papain, fucoidan and metoclopramide groups respectively. The result showed no significant difference across groups($p>0.05$) (figure 1).

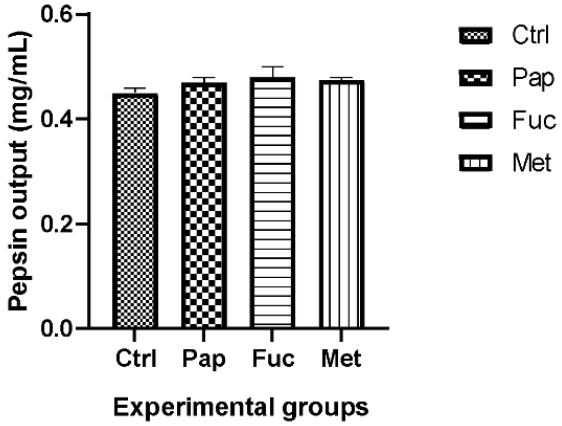


Fig 1: Comparison of pepsin output across control group and test groups
(Ctrl = Control, Pap = Papain, Fuc =Fucoidan, Met = Metoclopramide)
Values are expressed as mean ± SEM, n=5
There is no significant difference in pepsin output across the various groups

Comparison of mucus secretion across control group and test groups

The mean values of mucus secretion were 0.0800±0.00, 0.125±0.0100, 0.145±0.0100 and 0.160±0.00 for control, papain, fucoidan and metoclopramide respectively. The result showed a significant ($p<0.05$) increase across groups compared

to control as well as an increase in Met group compared to Pap group (figure 2).

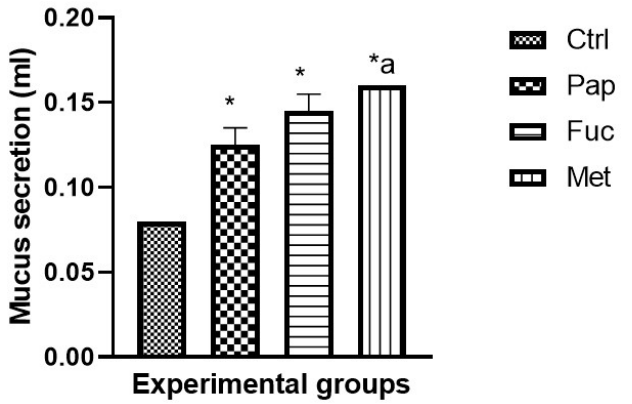


Fig 2: Comparison of mucus secretion across control group and test groups
(Ctrl = Control, Pap = Papain, Fuc =Fucoidan, Met = Metoclopramide)
Values are expressed as mean ± SEM, n=5
*=p<0.05 vs Ctrl, a=p<0.05 vs Pap

Comparison of ulcer score across control group and test groups

The mean values of ulcer score were 9.25±0.570, 14.1±0.360 11.5±0.460 and 12.7±0.260 for control, papain, fucoidan and metoclopramide respectively. The result showed a significant (p<0.05) increase across groups compared to control as well as a decrease in Fuc and Met group compared to Pap group (figure 3).

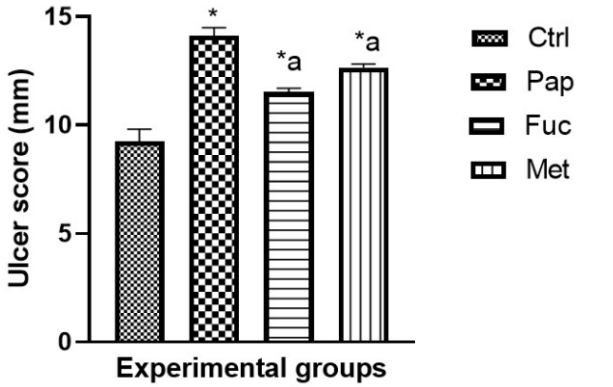
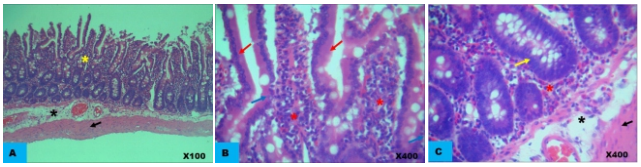


Fig 3: Comparison of ulcer score across control group and test groups
(Ctrl = Control, Pap = Papain, Fuc =Fucoidan, Met = Metoclopramide)
Values are expressed as mean ± SEM, n=5
*=p<0.05 vs Ctrl, a=p<0.05 vs Pap

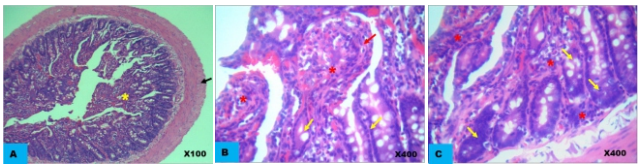
Histological results of the duodenum

Control group



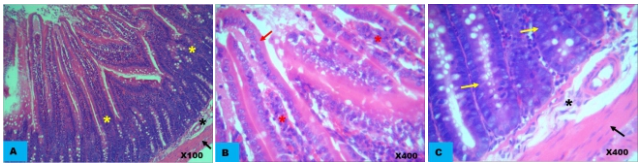
Sections of the duodenum showing normal histology. Plate A shows mucosa (yellow asterisk), submucosa (black asterisk), and muscularis propria (black arrow). Plate B shows the superficial mucosa with villi having enterocytes (red arrow), goblet cells (blue arrow), and lamina propria (red asterisk). Plate C shows the deep mucosa with intestinal glands (yellow arrow) and lamina propria (red asterisk), submucosa (black asterisk), and muscularis propria (black arrow).

Papain group



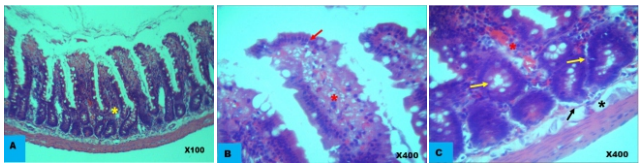
Sections of the duodenum show severe degenerative changes at the mucosa (yellow asterisk), and hypertrophy of the muscularis propria (black arrow) in Plate A. Plate B shows severe degeneration of villi with enterocytes necrosis (red arrow), atrophy/necrosis of intestinal glands (yellow arrow), and infiltration of lamina propria (red asterisk). Plate C shows severe necrosis of intestinal glands (yellow arrow) and infiltration of lamina propria (red asterisk)

Fucoidan group



Sections of the duodenum show normal histology. Plate A shows mucosa with abundant villi (yellow asterisk), normal submucosa (black asterisk) and muscularis propria (black arrow). Plate B shows marked recovery of villi enterocytes (red arrow) and lamina propria (red asterisk). Plate C shows marked recovery of intestinal glands (yellow arrow). Also note the normal submucosa (black asterisk) and muscularis propria (black arrow).

Metoclopramide



Sections of the duodenum show atrophy of duodenal wall. Plate A shows mild recovery of the mucosa (yellow arrow). Plate B shows mild recovery of enterocytes of villi (red arrow) and sparse lamina propria (red asterisk). Plate C shows recovering intestinal glands (yellow arrow). Note the sparse lamina propria (red asterisk) and muscularis mucosae (black arrow). The submucosa (black asterisk) is also sparse.

Discussion

The possible mechanisms through which aggressive factors induce ulceration in the stomach tissue and gastrointestinal tract, including bleeding, perforation and obstruction, can be subdivided into local and systemic actions.[23] Several studies indicate that gastric mucosal damage and inception of ulcers are accompanied by dynamic variations in the production of inflammatory cytokines, endothelium-dependent cyclooxygenases, epidermal growth factor receptor, and structural proteins of the extracellular matrix.[24,25]

The capability of gastric tissue to combat damage induced by its own digestive enzymes or foreign irritants, generally designated 'mucosal defence', is characterised as the secretion of mucus, regeneration of the epithelial membrane, angiogenesis, and bicarbonate production.[26,27]

Fucoidan obtained from the brown seaweed, Fucus vesiculosus, is composed of fucose, sulfate and ash plus aminoglucose. Limu, wakame, fucus, and hijiki seaweeds are good sources of fucoidan. Nutraceutical products containing the purified forms of U- and F-fucoidan are reportedly effective immune enhancers.[28]

The most significant benefits of fucoidan pertain to its ability to strengthen the immune system via anti-viral,[29] anti-cancer,[30] liver protection and anti-inflammatory.[31] Recent studies report that fucoidan prevents ulcers caused by acetic acid and Helicobacter pylori.[32]

Fucoidan has been shown to have anti-ulcer activity that can help protect against gastric ulcers. Pepsin output was insignificant across groups in this research, maybe because fucoidan doesn't directly affect pepsin secretion, but it can inhibit pepsin's activity in the stomach and protect against ulceration.[33]

Studies have demonstrated that fucoidan can inhibit pepsin; this anti-peptic activity is a key aspect of fucoidan's potential role in protecting against gastric ulcers.[34] By inhibiting pepsin, fucoidan may help reduce the damage caused by stomach acid and pepsin, which can lead to ulcers. Fucoidan also appears to have other mechanisms for protecting the stomach lining, such as promoting the production of growth factors that aid in ulcer healing and possibly modulating inflammatory response.[19]

Fucoidan's effect on mucus secretion is often linked to its ability to modulate inflammatory responses. It can reduce the production of inflammatory cytokines and other mediators, which can contribute to mucus overproduction. Evaluation of mucus production in the fucoidan group was raised compared to the papain and control groups, but decreased compared to the metoclopramide group. This increase in mucus, particularly in the gut and airways, can play a role in protecting against inflammation, pathogens, and allergens.[19,21]

Increased Fucoidan concentration can increase the thickness and secretion of mucus in the gut, which acts as a physical barrier against harmful substances. Elevated levels of Fucoidan increase mucus secretion, thus trapping and helping eliminate pathogens, reducing the risk of infection.[35]

With respect to gastric ulcers, fucoidan has been shown to aggravate the thickness of the mucus layer in the gastric mucosa, thus providing a protective effect. Fucoidan may directly influence the activity and number of goblet cells, which are responsible for producing mucus. Fucoidan can inhibit the formation of gastric lesions, promote epithelial cell regeneration, and potentially disrupt the adherence of Helicobacter pylori, a common cause of ulcers; Fucoidan may also play a role in protecting the gastric mucosa from damage caused by oxidative stress, a factor contributing to ulcer formation.[34]

Papain, a protease found in papaya, can reduce gastric acid secretion but does not directly stimulate pepsin secretion.[36] While papain can aid in protein digestion by breaking down proteins into smaller peptides, it does not influence the production or release of pepsin. This report aligns with our findings of insignificant change in pepsin output.

Papain can increase mucus secretion, particularly in the context of the body's "weep and sweep" response to irritants or pathogens. This report correlates with

our results of increased mucus secretion in the papain group compared to the control group. This increase in mucus secretion is often associated with goblet cell hyperplasia, where the number of mucus-producing cells in the epithelium increases.[37]

The increased number of goblet cells leads to a higher rate of mucus secretion, which can be a part of the body's defence mechanism against irritants or pathogens.[38] Papain can play a role in both promoting and preventing gastric ulceration. It has been traditionally used to aid digestion and treat digestive ailments.[39]

Papain has shown promise in wound healing, including skin ulcers, due to its anti-bacterial and fibrinolytic properties. Papain can also exhibit anti-inflammatory effects, which could be helpful in managing ulcers by reducing inflammation in the gastric mucosa.[40]

Some studies suggest that unripe papaya extracts, which contain papain, can protect the stomach lining and reduce the severity of ulcers, but isolated papain, especially in high concentrations, can potentially damage the gastric mucosa and worsen ulceration.[41]

The role of papain in gastric ulceration is complex and depends on various factors, including the form of papain (isolated or as part of a whole extract), concentration, and the presence of other substances. While it may offer some digestive and wound-healing benefits, it is crucial to be aware of its potential to damage the gastric mucosa, especially when used improperly, as shown in the histological evaluation of the papain group.

Histological examination of the duodenum reveals a normal mucosa, submucosa, and muscularis propria. Superficial mucosa with villi having enterocytes, goblet cells, and lamina propria was seen to be normal. It also shows the deep mucosa with intestinal glands and lamina propria, submucosa, and muscularis propria in the control group. These structural arrangements were observed to have been distorted in the papain group, leading to a degenerative, hypertrophic, and infiltration of the layers of the duodenum, respectively. The histological evaluation of the fucoidan group was observed to be normal, while that of the metoclopramide group was also noticed to possess distortion leading to atrophy of the duodenal wall, sparse lamina propria, sparse muscularis mucosae, as well as submucosa.

Conclusion

Fucoidan has proven to be a potent gastro-protective substrate with few side effects owing to its phytochemical composition. Papain has also shown to be gastro-protective to some indices and aggravate other parameters. In comparison to control and metoclopramide (a standard drug), fucoidan is less toxic than papain.

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Declaration of conflict of interest
The authors declare no conflict of interest.

Authors' Contributions: A.P.U. conceived and designed the study, supervised the experimental work, and critically revised the manuscript for important intellectual content. O.U.E. and O.C.O. carried out the animal experiments and sample collection. A.I.O.-E. and U.S.U. assisted with laboratory analyses and data acquisition. O.M.C. performed the statistical analysis and contributed to data interpretation. V.A.E. participated in histological processing and interpretation of tissue sections. A.P.U. drafted the initial manuscript. All authors reviewed, approved the final manuscript, and agreed to be accountable for all aspects of the work.

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