

Biochemically Reinstated Gastric acid and H^+ / K^+ - ATPase Enzyme Neutralization and In-vitro Anti-ulcer Bustle of Marine Fishes *Lutjanus argentimaculatus* and *Epinephelus malabaricus* Tissue Extracts

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ABSTRACT

There are several factors that may induce ulcers in human beings such as stress, chronic use of anti-inflammatory drugs, etc. Though in most cases, the etiology of ulcer is unknown, it has generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism. Thus, the search for a safe anti-ulcer drug that optimizes these properties is continuing, and part of the search is the evaluation of medicinal plants for gastroprotective properties. Adverse effects related to use of the majority of pharmacological drugs, particularly with their long-term use. The most effective and non-toxic fish sources for ulcer treatment are fatty fish (like salmon, mackerel, and sardines) have shown promise for anti-ulcer treatment with little or no reported toxicity. In our study, we assessed for anti-ulcer activities of marine fish *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extracts on artificial gastric acid, acid neutralizing capacity (ANC), and the H^+ / K^+ -ATPase inhibition model. The *Lutjanus argentimaculatus* extract significantly reduced ANC to 6.2 while *Epinephelus malabaricus* was 4.83 at a concentration of 1500 mg as compared to 14.50 with standard Aluminium hydroxide + Magnesium hydroxide (500mg). While in H^+ / K^+ - ATPase inhibition activity, the *Lutjanus argentimaculatus* extract showed maximum percentage inhibition of 79.56% while *Epinephelus malabaricus* was 70.02% at the concentration 100µg/ml as compared to 99.90% with standard Omeprazole. On the basis of the results, we may conclude that the *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extracts may be considered as a sole source of novel antiulcer drugs.

Keywords: Anti-ulcer, ATPase, Defense mechanism, Inhibition activity, Omeprazole,\

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INTRODUCTION

A stomach ulcer is formed as a result of a violation of the physiological balance between aggressive and protective factors of the mucous membrane. It has a rupture of the gastric mucosa, penetrating the muscularis mucosa and extending more than 5 mm in diameter [Hansson, 2000]. When changes occur in the defense mechanisms of the stomach, it can cause changes in the gastric mucosa, which will eventually lead to erosion and then ulcers. Over the past 20 years, stomach ulcers have affected 5 to 10% of the world's people and become a serious public health burden [Lanas and Chan, 2017]. Long-term use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol consumption, tobacco smoking, pathogenic *Helicobacter pylori* infection (infectious agents), stress, prostaglandin levels, and antioxidant enzyme

activity lead to peptic ulcer disease [Khedher *et al.*, 2022]. Damage to the gastric mucosa with the formation of an ulcer is the most common chronic disease of the gastrointestinal tract [Azmatullah *et al.*, 2022]. There is a link between chronic ulcers and stomach cancer. Adverse effects related to use of the majority of pharmacological drugs, particularly with their long-term use [Ebada *et al.*, 2020]. Significantly safer and no less effective in the treatment of diseases of the gastrointestinal tract can be the use of natural medicine. The most effective and non-toxic fish sources for ulcer treatment are fatty fish (like salmon, mackerel, and sardines) have shown promise for anti-ulcer treatment with little or no reported toxicity. In this regard, it is acknowledged that the problem of developing low-toxic and highly effective natural

preparations for the treatment of pathologies associated with inflammation and damage to the gastric mucosa is recognized as relevant.

In a broader sense, seafood makes up a substantial amount of the world's food output and comprises shellfish and finfish from freshwater, brackish, marine, and estuarine environments (Nag *et al.*, 2022). Shrimp shells, fish bones, fins, skins, intestines, and carcasses are among the many waste materials produced by the seafood industry in recent years, in addition to a significant amount of wastewater effluents (Nag *et al.*, 2022). Sardine oil may be a good natural source for the prevention and treatment of gastric lesions since it contains a high percentage of n-3 polyunsaturated fatty acids and has strong anti-ulcer properties (Vishnu *et al.*, 2017). In cases of ethanol-induced stomach ulcers, fish oil can lower gastric acidity and the gastric ulcer index, according to the Parham *et al.*, report, 2024. In both humans and animals, stomach injury is a prevalent condition. Gastritis and gastric ulcers are primarily caused by the disruption of the stomach mucosal barrier (Parham *et al.* 2024). Compared to synthetic anti-ulcer medications, many natural medicines exhibit potent efficacy against this illness without serious side effects (Singh *et al.*, 2023). Fish oil contains polyunsaturated fatty acids (PUFAs), which have anti-ulcer properties, however they are likely less effective than the anti-ulcer medications now on the market (Arumugasamy *et al.*, 2015). The present study was conducted to evaluate the in vitro anti-ulcer activity of marine fish *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extract on artificial gastric acid, acid neutralizing capacity (ANC), and the H⁺/K⁺-ATPase inhibition model.

MATERIALS AND METHODS

Collection of fishes

Fishes of the species *Lutjanus argentimaculatus* and *Epinephelus malabaricus* were gathered (Figure 1 and 2) from Nagapattinam on the Tamil Nadu East Coast in South India. The captured fish were inspected by looking at their eye brightness, gill color, and muscle texture. The identified fish sexes and according to their overall length for identification purposes, as reported by Fischer and Bianchi (1984) and Munro (1955). A maximum of ten fish per species were gathered, and the liver and muscle tissues were combined for examination. Personnel wearing latex gloves must handle collected fish and store them in sterile, clearly marked plastic bags. Additionally, fish must be dissected within three hours of being caught.



Figure 1 *Lutjanus argentimaculatus*



Figure 2 *Epinephelus malabaricus*

Preparation of homogenate

Each fish had its muscles removed, dissected, and cleaned with ice-cold physiological saline. A Teflon homogenizer was used to weigh and homogenize the 1g of tissues (10%). To assess antiulcer properties, tissue homogenate was made in 0.1 M Tris Hcl buffer (pH 7.4).

Preparation of artificial gastric acid

The artificial gastric juice was prepared according to the simulated gastric fluid test solution by the United States Pharmacopeia (USP) (Zagnoli, 1997). Two grams of sodium chloride and 3.2 mg of pepsin were dissolved in 7 ml of concentrated hydrochloric acid (12 M) and diluted with deionized water to make a 1 liter solution of the artificial gastric juice at pH 1.2. The artificial gastric juice was stored at 4°C until further use.

Determination of the neutralizing effect of extract on artificial gastric acid

Fifty milliliters (50 ml) of each test, water and standard solution was added to 55 ml artificial gastric juice at pH 1.2. The resulting pH value were determined to examine the neutralizing effects on artificial gastric juice. Sodium bicarbonate (1 mg/mL) used as a standard and pH value was determined by digital pen type pH meter.

Acid neutralizing capacity of extract

The different concentration of extract that can neutralize acid is 100mg/ml, 500mg/ml, 1000mg/ml, and 1500mg/ml. Magnesium hydroxide and aluminum hydroxide (500 mg) have been compared for the standard. The volume was 70 ml after 5 ml of the sample and the remaining 65 ml of water were added. This was blended for one minute. The 30ml of

1.0 N HCl was added to the standard and test sample and swirled for 15 minutes. Then, phenolphthalein was added and combined. The surplus HCl was promptly titrated until the pink color was achieved using 0.5N sodium hydroxide [Garad *et al.*, 2012]. The moles of acid neutralized is calculated by, Moles of acid neutralized = (vol. of HCl × Normality of HCl) - (vol. Of NaOH × Normality of NaOH) Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized divided by Grams of Extract / Antacid.

Preparation of H⁺ /K⁺ - ATPase Enzyme

The fresh goat stomach was purchased from the market butcher shop and used to make the H⁺/K⁺-ATPase enzyme sample. The gastric mucosa of the fundus was cut off and opened, and the inner layer of the stomach was scraped out for the parietal cell. The stomach parietal cell was homogenized in 16 mm .Tris buffer with a pH of 7.4, 10% Triton X100, and centrifuged at 6000 rpm for 10 minutes. The supernatant solution was then used to assess the H⁺/K⁺-ATPase activity.

Assessment of H⁺ /K⁺ ATPase inhibition

The reaction mixture of the sample containing 0.1 ml of enzyme extract (300 g) and plant extract at various concentrations (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml) was pre-incubated for 60 min at 37 °C. 2 mm ATP was added as the substrate, along with 200 ml each of 2 mm MgCl₂ and 10 mL each of KCl, to start the reaction. After 30 minutes at 37 degrees Celsius, the reaction was halted with 4.5% ammonium molybdate. Then, 60% perchloric acid was added, and the mixture was speed at 2000 rpm for 10 minutes to collect the supernatant containing liberate the inorganic phosphate, which was then detected at 660 nm using the Fiske-Subbarow (1925) method. 1ml of supernatant, 4ml of Millipore water, 1ml of 2.5% ammonium molybdate, and 0.4ml of ANSA were added after 10 min at room temperature. At different extract dosages, absorbance at 660 nm inorganic phosphate has been measured; the enzyme activity has been estimated as micromoles of Pi released per hour was compared to the well-known anti-ulcer PPA inhibitor Omeprazole. Results were expressed as Mean ± SEM [Gupta *et al.*, 2013]. % enzyme inhibition has calculated using the formula:

Percentage of inhibition = [Activity (control) - Activity (test)/Activity (control)] × 100

RESULTS AND DISCUSSION

Numerous health advantages of fish oil (FO) have been demonstrated, such as alleviating symptoms of multiple sclerosis, ulcerative colitis, rheumatoid arthritis, lupus erythematosus, psoriasis, Crohn's disease, and migraine (Maroon and Bost, 2006; Simopoulos, 2002). The n-3 polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid

(DHA) and eicosapentaenoic acid (EPA), are rich in fish oil (FO). FO successfully stops stomach ulcers caused by ethanol. The regulation of the stomach system was linked to the protective properties of FO (Mirghaed *et al.*, 2024). The present finding carried out the in vitro anti-ulcer activity of marine fish *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extract on artificial gastric acid, acid neutralizing capacity (ANC), and the H⁺/K⁺-ATPase inhibition model. Figure 1, shows the determination of the neutralizing effect of extract on artificial gastric acid. When different concentrations of extract 100, 500, 1000, and 1500mg (each 90 ml) were added to 100 ml of the artificial gastric juice (pH 1.19) separately, the pH values of *Lutjanus argentimaculatus* extract was found to be 1.40, 1.52, 1.62 and 1.80 while *Epinephelus malabaricus* 1.38, 1.43, 1.61 and 1.75. The pH values of water and Sodium bicarbonate solutions were 1.35 and 1.79 respectively. This results indicates that fish extract possess acid neutralizing property is dose dependent manner.

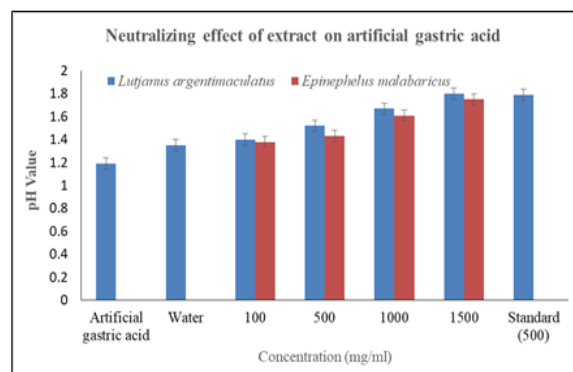


Figure 1: Determination of the neutralizing effect on artificial gastric acid of *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extract

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The *in-vitro* acid neutralizing effects of extract in different concentrations (100 mg, 200mg, 500mg, 1000 mg and 1500 mg per ml) were compared with the standard antacid AHMH- 500 mg/ml. The results showed concentration dependent reduction in acid neutralizing capacity per gm of antacid was found to be 83.5, 27.3, 10.8 and 6.2 for *Lutjanus argentimaculatus* extract while 72.30, 14.46, 9.95 and 4.83 for *Epinephelus malabaricus* extract. As Similarly, AHMH (500 mg) which is found ANC value 14.50 quite similar concentration of test drug. Whereas, extract concentrations 1000 and 1500mg were found to neutralize acid more significantly as compared to standard (Figure 2).

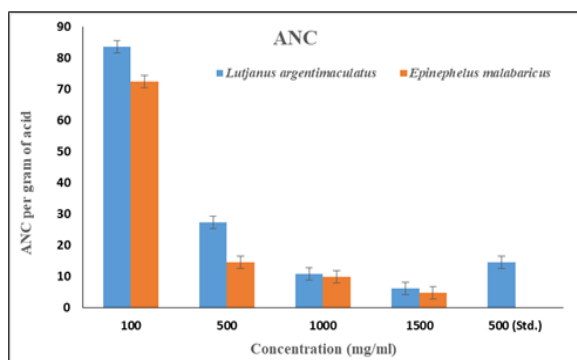


Figure 2: Determination of the Acid neutralizing capacity (ANC) of *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extract

$H^+ - K^+$ ATPase is an essential enzyme in promoting acidity (Yadav et al., 2012). The capacity of *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extracts to inhibit $H^+ - K^+$ ATPase in vitro was investigated in this study. Concentrations of the extract (20 μ g/ml, 40 μ g/ml, 60 μ g/ml, 80 μ g/ml, and 100 μ g/ml) were compared to those of the reference medicine Omeprazole (20 μ g/ml, 40 μ g/ml, 60 μ g/ml, 80 μ g/ml, and 100 μ g/ml). Inhibition of $H^+ / K^+ - ATPase$ activity by both test and standard drugs was found to be concentration dependent, with results ranging from 26.43 to 79.56% for *Lutjanus argentimaculatus* extract while *Epinephelus malabaricus* extract ranging from 20.98 to 70.02% and from 19.95 to 99.90% for the standard drug at 20, 40, 60, 80 and 100 micrograms per millilitre. $H^+ / K^+ - ATPase$ activity was observed to be inhibited by the extracts at concentrations higher doses were nearest to the standard Omeprazole (Figure 3).

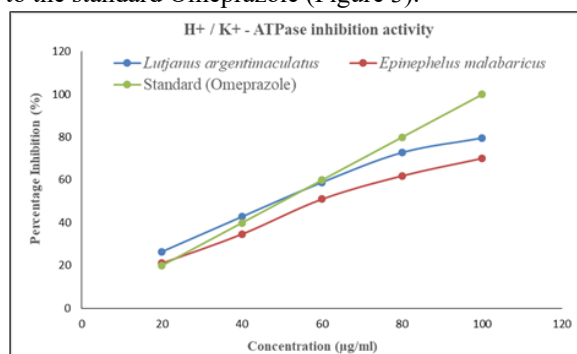


Figure 3: Effect of *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extract on H^+ / K^+ ATPase inhibition activity by in vitro

Fish oils that are high in polyunsaturated fatty acids (PUFA) have been shown to prevent dexamethasone-induced gastric damage (Manjari and Das, 2000). Fish oils that are high in vitamin E have been shown to prevent indomethacin-induced gastric damage (Zaror-Behrens et al., 1991). Fish collagen from catfish has been shown to have anti-ulcer effects (Sreerekha et al., 2016). The antioxidant capability of

fish collagen peptides can prevent stomach tissue damage caused by oxidative stress (Vijayan et al., 2012). Grinshpan et al., (2008) state that as the pH is directly measured when the sample solution is added to a fixed volume of artificial gastric acid, the neutralizing impact on artificial gastric juice can be utilized as a gauge of the antacids' commencement of action. It is a crucial component that needs to be considered when assessing antacid potential because one need for the perfect antacid is that it must react quickly with acids. Extracts from *Epinephelus malabaricus* and *Lutjanus argentimaculatus* demonstrated strong neutralizing effects. However, the *Lutjanus argentimaculatus* extract had a stronger neutralizing effect. These findings align with the extracts' ability to neutralize acid.

The essential membrane protein in charge of gastric acid secretion is the gastric $H, K - ATPase$, which belongs to the P2-type ATPase family. The special class of drugs known as proton pump inhibitors inhibits this enzyme, enabling the treatment of acid-related illnesses (Shin et al., 2009). Results from the PL model indicate that *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extract showed significant gastroprotective effect, through $H^+ / K^+ ATPase$ inhibition (Figure 4). The mechanism of action of *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extract underlying the decrease in H^+ , $K^+ - ATPase$ activity. However, the *Lutjanus argentimaculatus* extract had a stronger in vitro inhibitory effect on $H^+ / K^+ ATPase$ assay. The acquired results thus offer fresh insight into the omega-3 fatty acids component composition of *Lutjanus argentimaculatus* and *Epinephelus malabaricus*, and they imply that the examined extract can be used to develop fish medicines with antiulcer activity..

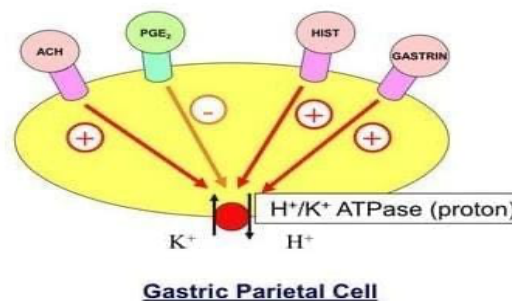


Figure 4. Proton pump inhibitors mechanism

Earlier reports by Ahmad Khusairi Azemil et al., (2018) examined the antiulcer activity of methanol-chloroform extract of *Channa striatus* fish. He shown that extracts from *Channa striata* are rich in essential

amino acids (like glycine and arginine) and polyunsaturated fatty acids that promote tissue repair, enhance collagen production, and possess antioxidant and anti-inflammatory properties. Studies have shown it significantly reduces the number and severity of induced gastric ulcers by augmenting defensive mucosal factors and inhibiting oxidative stress. It has also demonstrated potential inhibitory effects on the *H. pylori* bacteria, a common cause of ulcers

CONCLUSION

Along with free radical scavenging activity and antioxidant activity, H^+ , K^+ -ATPase inhibition was also assessed. The human stomach is found with the numerous gastric pits from which acid get secret. One of the cells, which lining the gastric pits is parietal cell, which is responsible for the acidification of stomach. PPA, the proton pump present in parietal cell is responsible for acid, which locates in the gastric membrane vesicle and actively transports protons into the lumen of stomach with the hydrolysis of the cytoplasmic ATP. Hyper secretion of this enzyme leads to acidity and ulcer. Presently, ulcer is treated by providing allopathic PPIs, antioxidants, H_2 -receptor antagonist etc., but these are associated with side-effects. The fishes *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extracts on acid neutralizing capacity and H^+ / K^+ ATPase inhibition activities were investigated. The results showed that the *Lutjanus argentimaculatus* and *Epinephelus malabaricus* extracts considered as a source of novel antiulcer and antacid drugs. And also *ajac* extracts possess an antacid and antiulcer property which may be due to the presence of biologically active antiulcer compounds in the extracts.

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