THERAPEUTIC EFFICACY OF POLYUNSATURATED FATTY ACIDS AND NUTRACEUTICALS: INTERVENTIONAL TREATMENT FOR INDUCED ULCERS IN ANIMALS AND IN CLINICAL STUDIES

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by

K. ARUMUGASAMY., M.Sc.,

Under the Guidance of
Prof. Dr. MANOJ G TYAGI, Ph.D,
at

INSTITUTE OF PHARMACOLOGY
MADRAS MEDICAL COLLEGE,
CHENNAI – 600 003
TAMILNADU, INDIA

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ABBREVIATIONS

AA	Arachidonic acid
ALA	Alpha linolenic acid
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATP	Adenosine trisphosphate
BMD	Bone mineral density
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
COX	Cyclooxygenase
CoA	Coenzyme A
CPCSEA	Committee for the Purpose of Control and supervision of experimental animals
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DGLA	Di-homo-gamma-linolenic acid
DPA	Docosapentaenoic acid
EFA	Essential fatty acids
EGF	Epithelial growth factor
EPA	Eicosapentaenoic acid
FO	Fish Oil
GLA	Gamma linolenic acid
GP_X	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidised glutathione
GST	Glutathione-S-tranferase
GT	Glutamyl transferase

HMG CoA	3-hydroxy-3-methylglutaryl coenzyme A
IAEC	Institutional Animal Ethics Committee
HETE	Hydroxyeicosatetraenoic acid
HNF	Hepatic nuclear factor
HPETE	Hydroperoxyeicosatetraenoic acid
IFN	Interferon
IGF	Insulin-like growth factor
IL	Interleukin
LA	Linoleic acid
LTB	Leukotrienes
LOX	Lipooxygenase
LXR	Liver X receptors
MDA	Malondialdehyde
mg	Milligram
Mg^{2+}	Magnesium ion
µg	Microgram
min	Minutes
ml	Millilitre
mm	millimeter
µmol	Micromol
mM	Millimolar
NF-kB	Nuclear factor kB
NSAIDs	Nonsteroidal antiinflammatory drugs
iNOS	inducible nitric oxide synthase
n-3	Omega 3
n-6	Omega 6
PBMC	Peripheral blood mononuclear cells
PGE	Prostaglandin
PGI	Prostacyclin
PIF	Proteolysis-inducing factor
PLA2	Phospholipase A2
PPAR	Peroxisome proliferator activated receptor

PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
RNS	Reactive Nitrogen Species
RXR	Retinoid X receptors
SREBP	Sterol regulatory element-binding protein
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TR	Thyroid hormone receptor
TX	Thromboxane
VEGF	Vascular endothelial growth factor

1. INTRODUCTION

The acid-peptic disorders presently in which pepsin and gastric acid are required, but when insufficient, leading to pathogenic factors. Pepsin and gastric acid in the stomach due to inherent defense mechanisms usually do not produce damage. The esophageal protection is by the barriers reflux preventing gastric contents into the esophagus. It may cause dyspepsia or erosive esophagitis if reflux and barriers protection fails. For stimulating esophageal motility and to enhance the lower esophageal sphincter and to reduce gastric acidity we require pharmacotherapy. There are many factors protecting the stomach known as "mucosal defense," by the production of prostaglandins and NO(1). If any disturbances to protecting factors, may cause a gastric or duodenal ulcer. The aim of antiulcer drugs is to decrease gastric acidity and enhance mucosal protection. *H.pylori*, an infective agent that contributes in the pathophysiology of peptic ulcer that has leads to new attitudes of treatment.

1.1 Functioning of Gastric Secretion:

It is continuous process in CNS and peripheral factors for the gastric acid secretion of H^+ by parietal cells. The factor that regulate acid secretion are by neuronal (acetylcholine, Ach), paracrine (histamine), and endocrine (gastrin). The receptors (H_2, M_3, & CCK_2) are situated on the basal and lateral surfaces of the parietal cells and some receptors on enterochromaffin-like cells (ECL), histamine is released from ECL. H_2 receptors are GPCRs that activate the G_s–adenylyl cyclase–C

AMP–PKA pathway. In parietal cells, Gastrin and Acetylcholine act via GPCRs, stimulates G_q–PLC-IP_3–Ca^{2+} pathway. The cAMP and the Ca^{2+}dependent pathway in the parietal cells activates H^+, K^+-ATPase, where it interchanges H^+ and K^+ ions. The pH of intracellular ~7.3 and pH of an intracanalicular ~0.8 is maintained by the proton pump. The gastric acid secretion is stimulated by the vagal nerve of dorsal motor nucleus, solitary tract nucleus and the hypothalamus. From the dorsal motor nuclei, the efferent fiber originates and goes down to the gut by the vagus nerve and synapse with the enteric nervous system's ganglionic cells. ACh promotes gastric acid production by binding with muscarinic M_3receptors on the basolateral membrane of parietal cell which is released from vagal postganglionic fiber. The CNS principally controls the action of the enteric nervous organization by ACh, in response to the, smell, sight, taste or anticipation of foodstuff .Ach motivates gastric acid production and release ("cephalic").

ECL cells typically are in contiguity to parietal cells and are the source of gastric histamine. Histamine acts as a paracrine mediator, diffuse from its location and act on the H_2 receptors of parietal cells. The important part of histamine secretion of gastric acid is significantly established via the ability of H_2 receptor inhibitors in diminishing the secretion of gastric acid.

The most potent inducer of secretion of acid is gastrin that is formed through antral G cells. Numerous pathways motivate release of gastrin, activation of central pathways, local distention and chemical constituents of the contents of gastric acid. Gastrin stimulated acid discharge proposes the production of histamine by ECL cells, an effect directly on parietal cell.

Somatostatin (SST) that is created by means of antral D cells hinder secretion of gastric acid. Acidity of gastric luminal pH toward <3 stimulate the release of SST, which further suppresses gastrin and liberate into a negative response loop. SST-producing cell is reduced in patients by *H. pylori* infection, and subsequently decrease of SST's inhibitory added to surplus manufacture gastrin.

Gastric Defenses against Acid:

Particularly extreme amount of H^+ in lumen of G.I tract requires defense mechanisms against the acidity to keep the esophagus and the stomach epithelial cells intact. The barrier reflux mechanism which prevents gastric acid contents into the esophagus is prevented by lower esophageal sphincter. The stomach defends itself from the damage by acid by a variety of mechanisms to involve sufficient mucosal flow of blood, possibly for high metabolic action and oxygen necessities of the mucosa of gastric tissue. One of the most important is mucus layer secretion that protect gastric epithelial cell by trapping bicarbonate by the cell surface. Gastric mucus is soluble when secreted it becomes insoluble gel and coats stomach mucosal surface, it slows diffusion of ions, and by preventing the damage to the mucosa from pepsin. Prostaglandins PGE_2 and PGI_2, responsible for the production of mucus, which is directly inhibited when gastric acid secretion level is high in the parietal cells. The drugs like NSAIDS and ethanol they inhibit the production of prostaglandins and reduce secretion of mucus and dispose toward the growth of acid-peptic disorder.

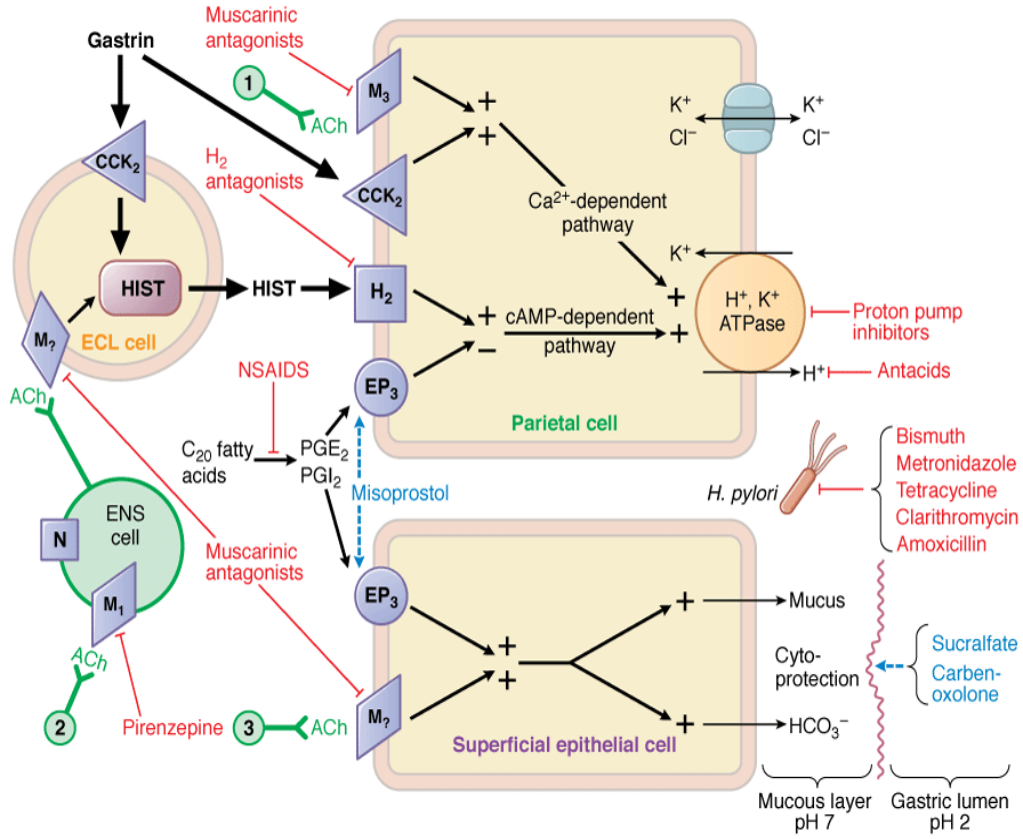


***Figure 1**: Physiological with pharmacological regulation of gastric secretion*

1.2 Peptic Ulcer Disease:

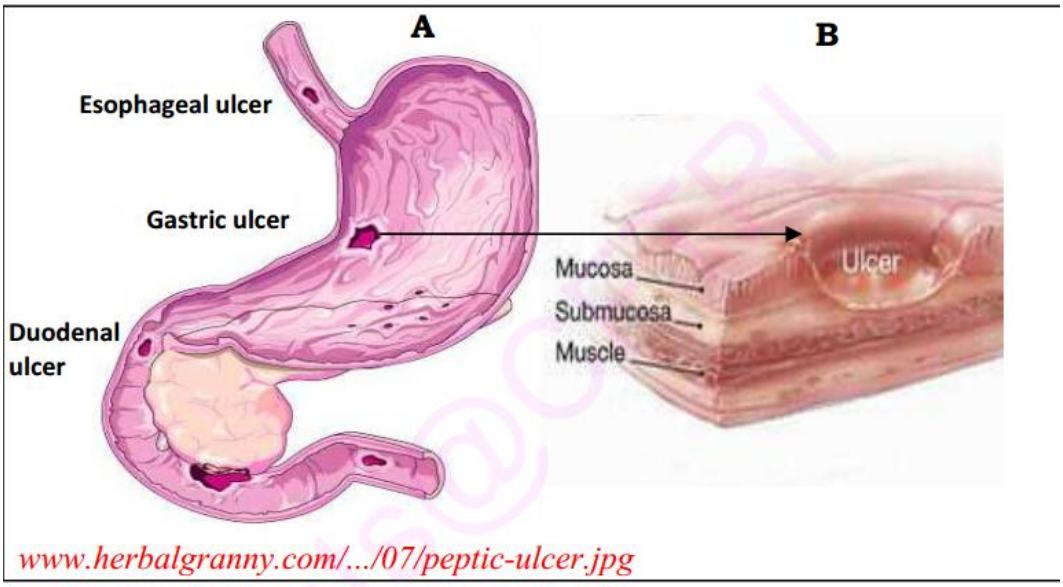
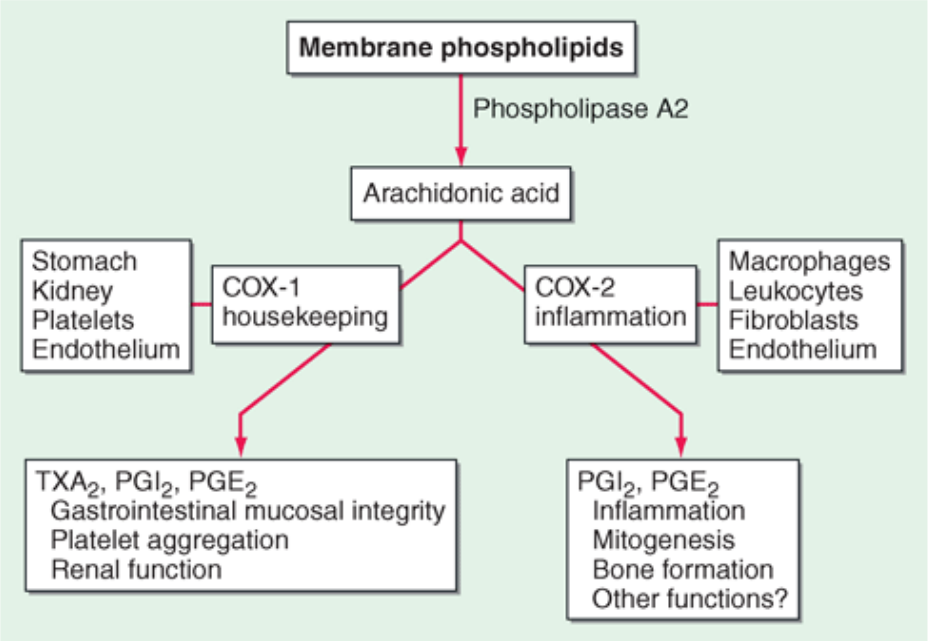


***Figure 2:** Picture showing esophageal, gastric and duodenal ulcers (A), penetrating deep into the mucosa and submucosal layer (B).*

The pathophysiology of peptic ulcer disease is most excellent view while an imbalance between mucosal cover factor (bicarbonate, mucin, prostaglandin, NO, other peptides and growth factors) with noxious factor (pepsin and acid). Going on average, patients among duodenal ulcers produce more acid than do be in command of subjects, mostly at night time (basal secretion). Though patients with gastric ulcers comprise normal or even reduced acid production, ulcers seldom but ever occur in complete deficiency of acid. Up to 60% of peptic ulcers are seen with H. pylori infectivity of the stomach. This illness may lead to impair formation of somatostatin all the way through D cells and, decrease in inhibition of gastrin production, as a result increase acidic production with reduce duodenal bicarbonate production.

NSAIDs are also frequently associated with peptic ulcers and bleeding. Topical injury by the luminal presence of the medicine appear to perform a negligible part in the pathogenesis of these ulcers, as evidenced by means of the fact that ulcer can occur with especially low dose of aspirin (10 mg) or by parenteral administration of NSAID (1). The special effects of these drugs are suppression of mucosal prostaglandin synthesis (particularly PGE_2 and PGI_2). Most of the mucosal prostaglandin synthesis occurs via the constitutively expressed cyclooxygenase-1 (COX-1), but COX-2, which can be very rapidly induced, also contributes significantly to the generation of mucosal-protective prostaglandins. Thus suppression of COX-1 and COX-2 by NSAIDs contributes to the induction of mucosal injury. COX-2-derived prostaglandins are particularly important in repair of mucosal injury (1).



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Figure 3: ***Graphical depiction of the stages participating in production of prostaglandin E2 (PGE2) and prostacyclin (PGI2).Uniqueness and distribution of cyclooxygenase (COX) enzymes 1 and 2 are likewise presented. TXA_2, thromboxane A_2***

1.3 Causative factors of ulcer pathogenesis:

Ulcer is a multi-factorial induced disease. The Pathogenesis of ulcer, a multifactorial complication, has been studied over for more than numerous years. Ulcers are produced by an disparity of hostile gastric luminal elements like acid and pepsin and also joined with cover up injury from ecological or immunologic substances and protective mucosal barrier function (2). Some environmental as well as host causes add to ulcer development by increase in gastric acid release or by deteriorating the mucosal barrier (3). Apart from genetic factor, at least 5 other factors which are contributing to ulcer pathogenesis such as stress, use of NSAIDs, H. pylori infection, smoking and alcohol consumption (3).

Stress:

In the pathogenesis of ulcer, emotional stress and psychosocial causes are frequently recognized as substantial contributor (4).An excellent case of stress in the bleeding gastric ulcer in aged individuals after a brutal earthquake in Japan (5). The production of free radical are mainly implicated as a causative mechanism in stress induced ulcers (6).

NSAIDs:

Chronic use of NSAIDs is the subsequent most frequent reason of ulcer and the speed of NSAID-caused ulcer have been growing with reference to 20 million people receive prescription of NSAIDs regularly.

Food and beverages:

Some type of food along with beverages are reported to cause dyspepsia (7) also harmful effect of red and black pepper play role in secretion from parietal cells, secretion of pepsin, and loss potassium ion as well as gastric cell exfoliations with mucosal microbleeding, which are similar to those, induced by aspirin.

Ulcer pathogenesis:

The well defined mechanism of ulcer pathogenesis was established only in stress, NSAIDs and H. pylori induced ulcers and these are the major causative factors of gastric ulcer. Therefore the detailed mechanism of stress, NSAIDs and H. pylori induced ulcer pathogenesis is described as follows.

Stress induced ulcer pathogenesis:

Ulcer patients characteristically demonstrate the similar psychosomatic structure as the universal population, but they seem to observe a larger amount of stress. The consequences of most study designate that 75% to 100% of patients in the ICU include defect of the gastric mucosa in hours subsequent to admittance (8). The connection between serious physiologic stress and gastrointestinal (GI) ulceration is clearly recognized. The cause of stress-related mucosal disease (SRMD) has not been explained totally, although there is solid proof that hypoperfusion of the superior GI tract is the chief reason. Tough management of the causative disease is mainly the chief factor in the cure of stress ulceration (8). In a rat model, (9) found that oxygen derived free radicals, mainly O_2-, participate in an vital role in the synthesis of gastric lesions made by ischemia and hydrochloric acid during stress condition. Investigators have showed that an extended time of ischemia causes higher amount of lesions and that reperfusion (retransfusion) was an important step in the development of lesion. The word stress-related mucosal disease (SRMD) stand for a variety of situation range from stress-related damage to stress ulcers (focal deep mucosal damage) as shown in Fig 4 .Stress is identified to provoke oxidative stress caused by formation of free radicals leading to a) up regulation of H+, K+- ATPase; b) increase in acidity; c) disruption of mucosal epithelium and; d) susceptibility for H. pylori infection ultimately leading to e) gastric ulceration as depicted in Fig 5 & 6.

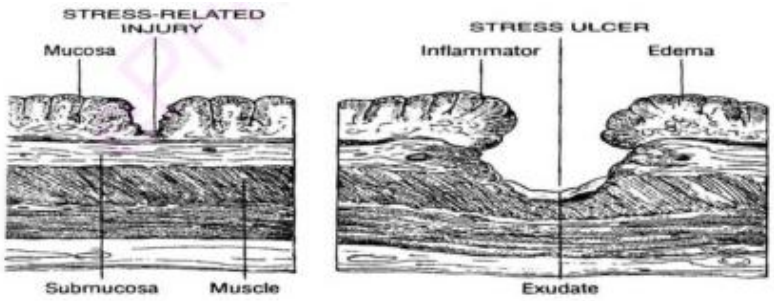


Figure 4: Depth of tissue injury in stress –related injury (A) and stress ulcer (B).

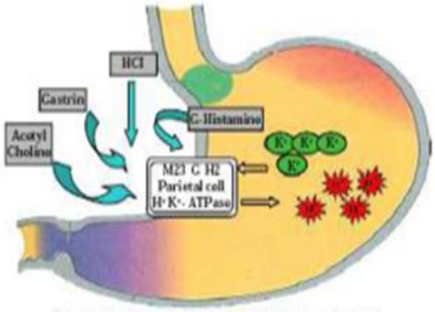


Figure 5: Disregulation of parietal cell activity results in hyperacidity during stress

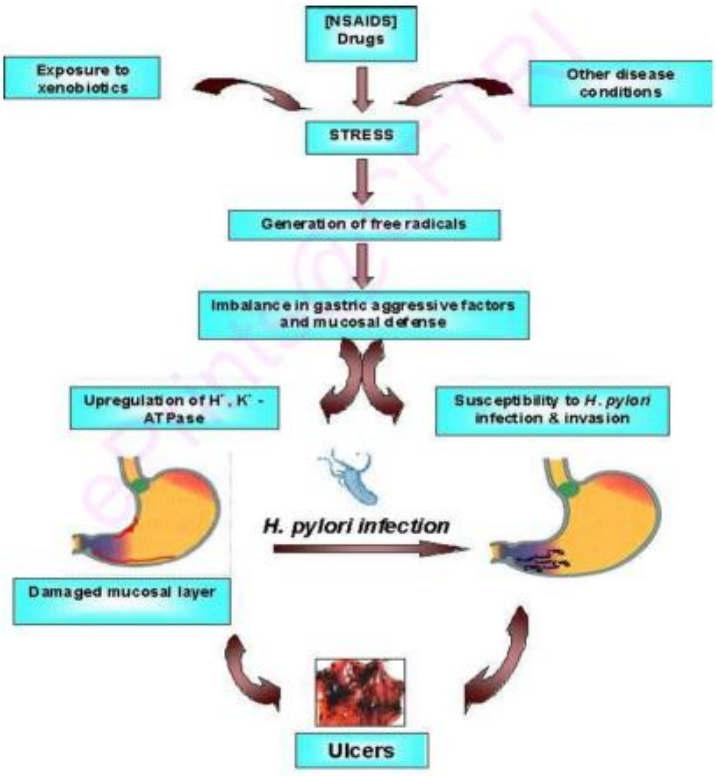


Figure 6: Multi-steps involved in stress induced ulcer pathogenicity.

Ref: Dharmesh & Srikanta, 2009.

NSAIDs induced ulcer pathogenesis:

The most frequent NSAIDs are aspirin, ibuprofen, and naproxen, although many others are available (Fig 6). The notion of gastroduodenal mucosal damage has developed commencing the idea of topical damage to concept that entails numerous processes of mucosal defense. Superficial injury by trapping of ions (10) and decrease of mucus gel hydro-phobicity (11) was formerly considered to be a vital mechanism of NSAID-induced gastric injury.

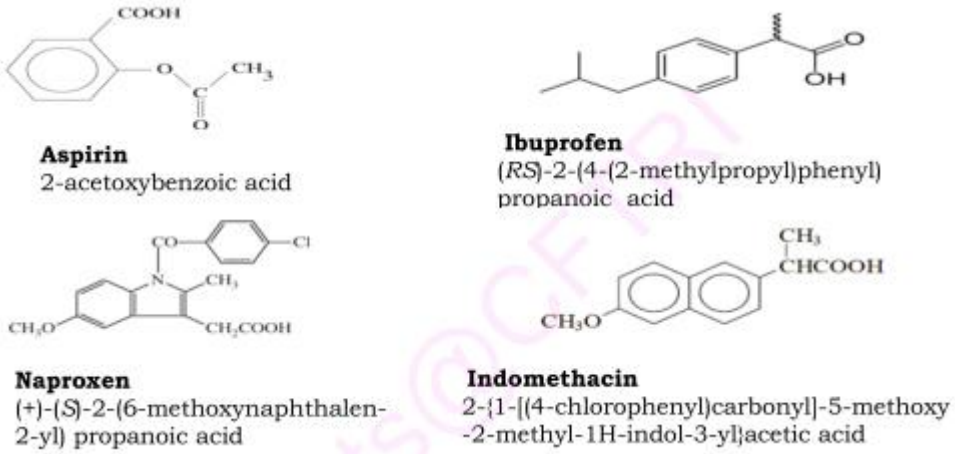


Figure 7: Structures of most commonly used NSAIDs

Since Sir Vane's finding in 1971(12) that, NSAIDs harm stomach mostly by suppression of gastric prostaglandin formation, here is considerable facts to facilitate the ulcerogenic consequence of an NSAID correlate well with its aptitude to repress prostaglandin formation. The study by Wallace and colleagues show to facilitate selective inhibition of whichever COX-1 or COX-2 is not connected by means of gastrointestinal damage. Rather, it was suggested to be the double inhibition of COX-1 and COX-2 that is essential. Selective inhibition of COX-2 slow down the

healing of investigational ulcers, suggestive of COX-2 importance in re-establishing of gastric mucosal structure (13) . Neutrophil adherence destroys the mucosa by releasing oxygen free of charge radicals, release proteases, in addition to hinder capillary blood flow in NSAID user as shown in Fig 7. Prevention of neutrophil adherence lessens NSAID mediated damage in rodent models. On the other hand, there are number of studies showed the necessity of prostaglandins, particularly PGE_2 in mucoprotection (14). Prostaglandins arouse several factors feel to be important here maintaining normal mucosal integrity, such while mucus synthesis and secretion, mucosal bicarbonate secretion, mucosal blood flow, and cellular repair (15) as a result systemic consequence of NSAIDs seem to show a major function through the reduced production of mucosal prostaglandins by inhibition of COX enzyme (16) (Fig:9).

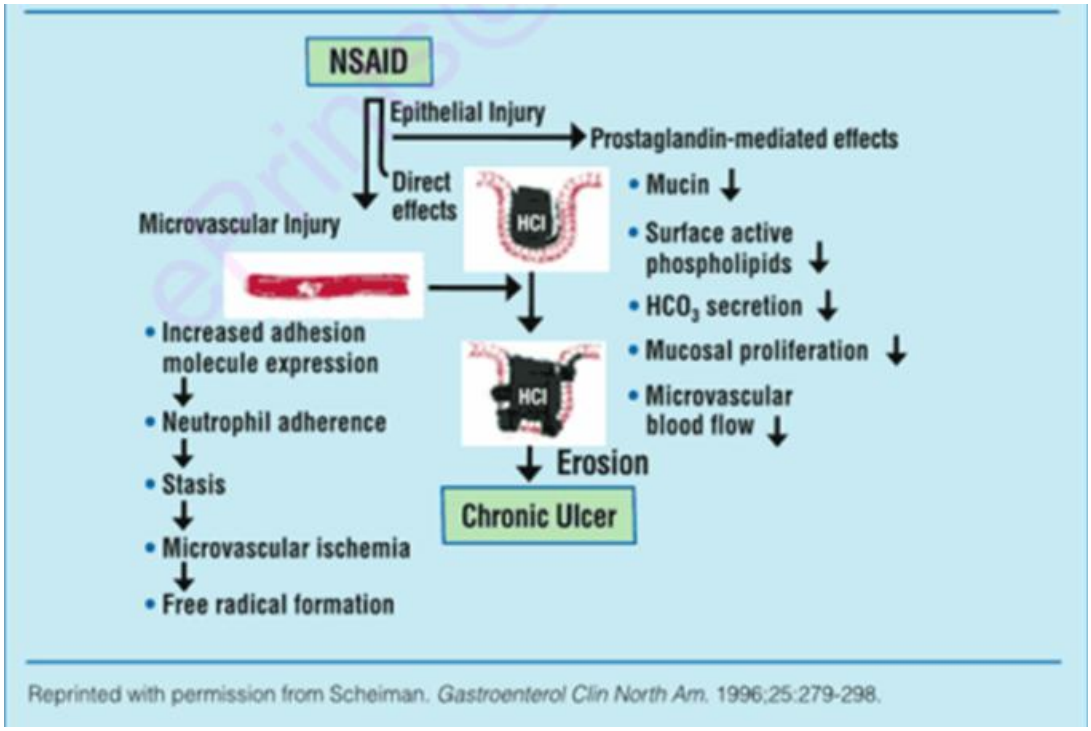


Figure 8: COX inhibition and GI Toxicity.

In addition, two methodical review have shown to H. pylori infectivity substantially increase risk of peptic ulcer and bleeding from ulcers in long term NSAID users due to combinational effects as shown in (Figure 8) (17). Therefore, although mucin protection and acid suppressions are the core of NSAID-associated ulcer disease management, H. pylori eradication is needed in case of H. pylori positive ulcers.

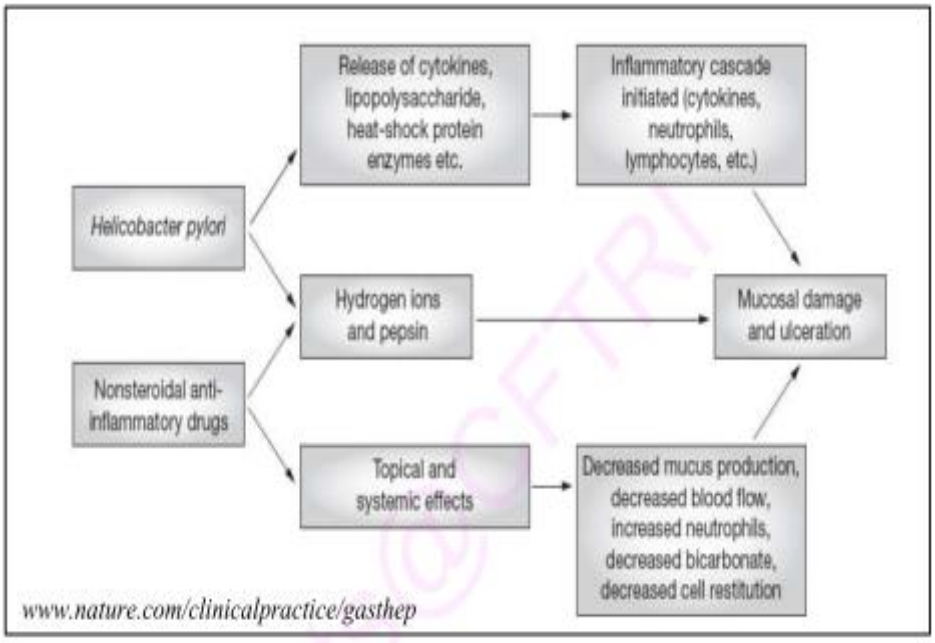


Figure 9: ***NSAIDS and H.pylori act synergistically through inflammatory pathways to develop gastric ulcers.***

H. pylori mediated ulcer pathogenesis:

Marshall and Warren's (Fig 10 A) seminal discovery that a bacterium, H. pylori (Fig 10 B), causes gastric ulcers, and in some cases, gastric cancer, merit the 2005 Nobel Prize in Physiology or Medicine in favor of its remarkable impact happening public health and meant for opportunity up latest avenues of research.

Amongst the more than 20,000 articles on H. pylori published ever since 1983, many have been devoted to the study of the bacterium and understanding its secrets (18). Member of the genus Helicobacter be all microaerophilic organisms as well as in the majority of cases be catalase and oxidase positive, with many however not all species are also urease positive.

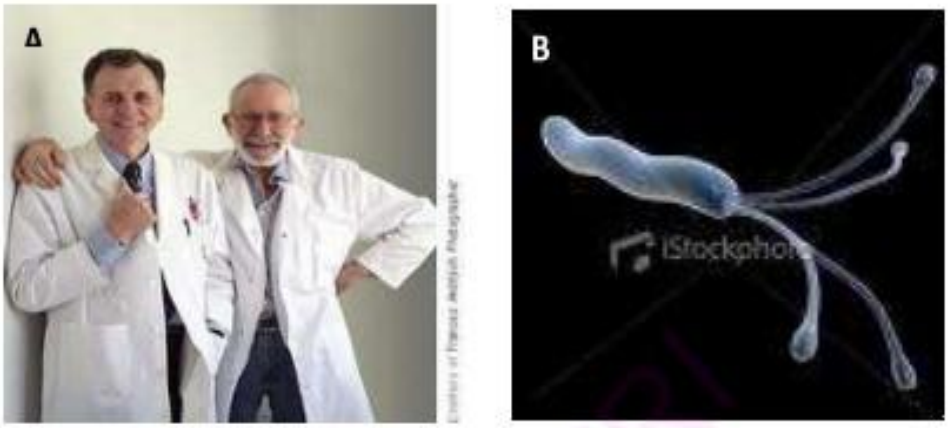


Figure 10: ***Barry Marshall (left) and Robin Warren (right) in Fig A and image of Helicobacter pylori (B)*****:** Winners of 2005 Nobel Prize meant for their finding of "the bacterium Helicobacter pylori along by its role in gastritis and peptic ulcer disease. Fig B is an image of H.pylori.

An important characteristic of H. pylori is its urease activity and microaerophilicity, with most favourable growth at O_2 levels of 2 to 5% and the supplementary need of 5 to 10% CO_2 and high moisture. Development happens at 34 to 40°C, by an optimal of 37°C.

Table 1: Regimens Recommended for Eradication of H.Pylori Infection.

Drug	Dose
Triple Therapy	
1. Bismuth subsalicylate*plus*	2 tablets qid
Metronidazole*plus*	250 mg qid
Tetracycline[a]	500 mg qid
2. Ranitidine bismuth citrate *plus*	400 mg bid
Tetracycline*plus*	500 mg bid
Clarithromycin or metronidazole	500 mg bid
3. Omeprazole (lansoprazole) *plus*	20 mg bid (30 mg bid)
Clarithromycin*plus*	250 or 500 mg bid
Metronidazole[b] *or*	500 mg bid
Amoxicillin[c]	1 g bid
Quadruple Therapy	
Omeprazole (lansoprazole)	20 mg (30 mg) daily
Bismuth subsalicylate	2 tablets qid
Metronidazole	250 mg qid
Tetracycline	500 mg qid

Commercially accessible drugs given for gastric ulcer therapy, has various unpredictable side effects which affects 5% of the global population (19), so it is a major drawback for the treatment regimen in modern times.

Now days, treatment given to reduce or prevent the side effects have become one of challenging problems of clinical medicines. Therefore it is necessary to discover a medicine having anti ulcer function with no adverse effects, which can acts as a dominant therapeutic instrument to heal gastric ulceration, so the hunt has been extends to the methodical improvement of natural products, from prehistoric era. Gastroduodenal ulcer disease consequences from an anomaly in the mucosal

barrier. Helicobacter pylori as well as NSAIDs are the significant reason of this malfunction of barrier function causing ulcer development. GI tract is a ulcer disease and is well attributed towards extreme release of hydrochloric acid quite than to a most important malfunction of the barrier itself (20).
In 1972, John Hunter drafted a hypothesis stating that there is inherent resistance of the stomach to autodigestion. On examining the speed of postmortem gastric autolysis, he attributed the capacity of the stomach not to be digested itself during life is due to the existence of a “living principle."

In Hunter’s view this “living principle." is the continuous circulation of blood through gastric tissue. In the year 1853, Virchow advanced this theory, stating that the acid in the gastric juice which diffused support into the mucosa, where it was deactivated by flowing alkaline blood. Gastric ulcers were measured to be secondary to a constraint in local blood supply, with consequential unproductive deactivation of absorbed acid, causing restricted areas of auto-digestion.

The significance of cytoprotection by prostaglandin-E_2 currently accepts substantial attention because of rising interest concerning NSAID-induced ulcers. Yet, these prostaglandins decrease gastric mucosal flow of blood, which hypothetically should be harmful. It have been approved that improved formation of mucus, along with hardening of the mucus layer, is the answer to prostaglandin-induced cytoprotection (20). Peptic ulcers are deficits in the gastrointestinal mucosa which expand throughout the muscularis mucosae. They continue as a function of the acid or peptic action in gastric juice. Peptic ulcer is an significant reason of morbidity with expenses in the United States ascribed to current ulcers projected at

$5.65 billion per year (21).Worldwide development of drinking of alcohol and non-steroidal anti-inflammatory drugs (NSAID) with improper eating habits have added in the direction of the rise in ulcer etiopathology (22). Through this method, the peptic ulcer is believed a disease of present era, connected to the addiction that is ever more frequent into the society as well as toward its taxing way of life. Dealing with natural food guarantees a cure. Plant life have been unprocessed substance for the production of a variety of drugs in addition to they continue to be an essential resource of novel restorative agents (23) presented a analysis to demonstrated the huge diversity of chemical materials isolated as of plants products to present anti-ulcerogenic activity, representing their huge prospective in finding of new treatments meant for ulcers.

Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and piroxicam and Indomethacin remain amongst the most frequently utilized pharmacological agents (24). Though, these groups of materials may source gastrointestinal ulcer formation, due to the capability of these drugs, to suppress prostaglandin production. Indomethacin is a preferential COX-1 inhibitor. Cyclooxygenase be constitutively articulated in the gastrointestinal tract into huge quantities and have been showed to preserve mucosal reliability through constant production of prostaglandins (25). Peptic ulcer disease, which is marked as an abrasion in the inside layer of the mucosa G.I bathed by acid and pepsin. It is the chief G.I disease (2), (26) with a global incidence rate of around 33% in the advanced nations and 50% in individuals living in the emerging nations due to infection caused by H. pylori. Peptic ulcer disease by is due to deficiency of balance between the gastric violent factors and the mucosal self-protective factor (27). Peptic ulcer is distinguished by damage to the

mucosa and is primarily originated by Helicobacter pylori or anti-platelet drugs for instance aspirin. Other contributory factors are drugs that belong to the class of non steroidal anti-Inflammatory Drugs (NSAIDs) and oral bisphosphonates, immunosuppressive medications (28), potassium chloride, serotonin reuptake inhibitors, cigarette smoking and alcohol consumption (29).

Ulcers induced by ethanol have been reported to show enormous morphologic and metabolic aberrations in the gastric mucosa of experimental animals related to those observed in the human being peptic ulcer (30). Administration of ethanol is recognized to make ulcerative lesion and raise lipid peroxidation in the gastric mucosa, that exists as a considerable element in the pathogenesis of mucosal lesions (31). Ethanol-induced oxidative damage is commonly attributed to the formation of the extremely reactive hydroxyl radical, stimulator of lipid peroxidation, leading to destruction of mucosal membrane (32).

These factors could be capable of reason submucosal erosion and hinder cyclooxygenase, thus troubling the defense of the gastric mucosal layer (33). According to anatomy, peptic ulcers arise regularly in the abdomen and proximal duodenum peptic ulcers because by an discrepancy in-between the defensive (mucosal barrier, blood flow, mucus secretion, endogenous protective agents) and destructive (pepsin and acid secretion) role of the gastric system (34). Gastric lesions induced by alcohol disturb gastric defense aspect such as mucus secretion and mucosal circulation (35). Necrotic lesion caused by ethanol in gastric mucosa in the course of numerous pathways, directly produce necrotic lesions, which consecutively reduce defensive factors, mucus production and bicarbonate

discharge. As a defensive barrier, the gastric wall mucus is believed to play a crucial role against gastrointestinal damage (36). Mucus secretion is regarded as to be a crucial defensive factor that protects the gastric mucosa from getting lesions (37). The gastric wall mucus levels has been assessed previously also to be used as sign of gastric mucus secretion (38). Huge number of medicinal plants has been reported by researchers, as a means of antiulcer properties. Plant-based drugs signify an immense available resource that has revealed massive therapeutic potential.

Indication of peptic ulcer disease include pain in abdomen, nausea, regurgitation, deficit of appetite and weight (39). This disease might also leads to hemorrhage and perforation in upper gastrointestinal tract (40)which can cause higher morbidity and rate of death. In most of the common cases, the production of Reactive Nitrogen Species (RNS) and reactive oxygen species (ROS) in the human abdomen (41), causes oxidative stress on the gastric mucosa (42), is increased by H. pylori infection.

NSAIDs can cause erosion of submucosal tissue and also and inhibit the enzyme cyclooxygenase, that decreases the production of prostaglandins and deteriorates the layers of gastric mucosa (43). The mixture of antacids, anti-secretory drugs, and antibacterial drugs has been recommended for the treatment of peptic ulcers. A combination of H_2 receptor antagonists such as ranitidine and famotidine (44), proton pump inhibitors (45) and clarithromycin, amoxicillin, or metronidazole helps as a regular typical therapeutic schedule. Even though several endoscopic as well as pharmacological treatment sexist for peptic ulcer disease, these managements of the disease condition usually display inadequate effectiveness in

opposition to gastric diseases, and are frequently related with mild adverse effects. In disparity, natural products have displayed efficient therapeutic properties with lessened adverse effects (46).Additionally, the pharmaceutical manufacturing companies are considering the benefits of herbal products.

Recent findings show significance of PUFAs innumerous clinical ailments. So, observing the consequence of PUFAs in several clinical conditions It was advised that insufficiency of PUFAs specifically gamma-linolenic acid, di-homo-gamma-linolenic acid, arachidonic acid & eicosapentaenoic acid might be accountable for the development of peptic ulcer (47- 48). PUFAs hold the capacity to obstruct the development of helicobacter pylori, decreases the acid formation and release in experimental animals, in individuals and also in the improvement of the liver situations like severe hepatitis C (49- 50). PUFAs can cure the ulcer and propose cytoprotective roles by amplifying PGE_1.

The main goal of this research is to investigate the anti-ulcer and ulcer-protective property of PUFA. The current work is devised to explore the anti-ulcer outcomes of polyunsaturated fatty acids in rats in which the ulcers were created experimentally.

2. REVIEW OF LITERATURE

2.1 Polyunsaturated Fatty Acids (PUFA):

Introduction:

Lipid intake in diet amount to 25%–45% of the total energy. Fatty acids are of great interest to biochemist. Fatty acids are obtained from dairy products, meat and oil giving raise to intake of monounsaturated and saturated fatty acids as well as comparatively modest amount of polyenes. Osteoporosis, cancer, inflammatory diseases, cardiovascular diseases, dyslipidemias and diabetes are all related to dietic factors. PUFA breakdown is engaged in vital for upkeep of biological homeostasis, eicosanoid metabolism. In order to modulate transcription of regulatory genes PUFA acts on nuclear receptor proteins which bind to a definite region of DNA. This new mechanism of action gives good understanding on metabolic effects of PUFA which helps in both future drugs and dietary components with very less side effects and useful effects.

Chemistry:

PUFA belongs to the category of simple lipids, containing unsaturated bonds in the *cis* configuration. The major family of PUFA is the Linoleic acid (n-6) (LA) and the alpha-linolenic acid (n-3) (LNA) which are as nutritional essential fatty acids (EFA) because it could not be manufactured by human beings and which helps to prevent many deficiency symptoms.

Sources:

LNA is found in chloroplast of green vegetables like linseed, purslane, spinach, walnuts and in seeds of flax . Docosahexaenoic acid (C22:6n-3, DHA) and eicosapentaenoic acid (C20:5, n-3, EPA) are the main source from fishes. LA, is discovered in huge amount in soyabean oil, corn oil, sunflower oil, and safflower oil (51).

Evolutionary:

Studies show that in the human beings diet the amount of n-6 and n-3 fatty acids are quite equal and were low in saturated fat. The progress of agriculture, made changes in food supply over the past 10,000 years. Last 100–150 years variations directed to rise in *trans*-fatty acids from hydrogenation of vegetable oil, escalation in saturated fats from grain-fed cattle, increase in n-6 fatty acids (about 30 g/day) owing to the manufacture of oil from safflower, corn, vegetable seed and cotton. Increase in meat consumption led to increased quantity of LNA is merely 2.92 g/day (52), and amount of arachidonic acid (C20:4n-6, AA), around 0.2–1.0mg/day(51), while the quantity of DHA, EPA are 72 and 42 mg/day. Quantity of n-3 fatty acids decrease directed to a disparity and growth in the percentage of n-6/n-3.

Recommendation:

Benefits with consumption of n-3 fatty acids directed to selling of products comprising these fatty acids. In Europe, on the basis of an report on "*the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy*",

minimum requirement of LA is 1% (53) and 0.2%– 0.5% (54),LNA of energy consumption. In the U.S., total PUFA intake should not exceed 10% and remain 7% of energy intake .In Japan, balanced intake of n-3 and n-6 PUFA is approximately 26%, but an increase in ratio is observed in young persons. Hence, education about nutrition is necessary.

PUFA Metabolism:

Essential Fatty Acid LA and LNA are present in our diet. In human beings 18-carbon precursor from highly unsaturated members namely AA and DHA. EFA metabolism, at first takes place in liver and further the process is continued in tissues also (55). The foremost quantity of the metabolic pathway to EPA (C20:4 n-6 and C20:5 n-3) and AA happens in endoplasmic reticulum and involves progressive substituting desaturation and elongation stages catalyzed by delta5- and delta6-desaturase and fatty acid elongase. Here rate limiting step takes place with the help of delta6-desaturase. Final conversion of DHA and 22:5n-6 is still not agreed. It has been thought it occurs by delta 4- desaturase (56), but still no proof of existence is being found (57). It is proved that final portion happens via desaturation and chain elongation and pursued by retro-conversion stage of peroxisomal beta-oxidation, so-called “Sprecherpathway”. Huszagh and Infante (58) the biosynthesis of DHA and 22:5n-6 occur by distinct channeled carnitine- facilitated mitochondrial pathways.

Effects:

Essentiality:

Both the fatty acids, LA and LNA are now regarded as nutritionally EFA. The symptom of EFA deficiency (dermatitis, infertility and growth retardation) relate to n-6 fatty acids (59). Organizational constituent in the ceramides of the water blockade of the skin is LA; precursors of eicosanoids are AA. In the process of signal transduction n-6 fatty acid plays a role. Inadequate LA intake causes protein energy malnutrition and fat malabsorption. Essentiality of n-3 fatty acids lags behind and can take part as additional for n-6 fatty acids, in some EFA deficiency symptoms. Natural task of dietary n-3 fatty acids in the organism (59) is to provide carbon atoms and energy. DHA and EPA serve as a precursor for "n-3 eicosanoids increasing role of DHA in neuron tissues and also in retina. n-3 PUFA deficiency can cause a loss of DHA from brain and retina-rod outer section phospholipids which is substituted by 22:5 n-6. This alteration in membrane phospholipid construction will lead to learning disabilities, memory damage and diminished visual perception.

Eicosanoid Metabolism:

Essential Fatty Acid in plasma membranes function as substrates for enzyme lipooxygenase (LOX) and cyclooxygenase (COX) are transformed into functioning, brief lived, hormone-like compound referred to as "eicosanoids." Synthesis of eicosanoids is influenced by its liberation from cellular supplies by phospholipase A_2 (PLA_2). Eicosanoid influences metabolic activity like clumping of platelet, edema, bleeding, vasoconstriction and vasodilatation, vascular resistance

and immune purposes. AA is the substrate for"series 2" leukotrienes (LTB_4, series B_4 LT), prostacyclins (PGI), prostaglandins (PGE), thromboxane (TX).

Excessive Alcohol Consumption:

Membrane structures are altered throughout alcoholism as it prevents phospholipase function. PUFA are too precursors of eicosanoids which supports in the control of blood pressure. Consequently extreme alcohol ingesting leads to hypertension and modifications in hepatic PUFA breakdown. Food accompanied along with n-3 PUFA enhances the synthesis of AA, probably by a delta-5 desaturation (60). Chronic alcoholics displayed that peripheral blood mononuclear cells (PBMC) synthesized fewer prostaglandin E2, and neutrophils synthesized.

Stress:

Gudbjarnason (61) presented in rats the recurrent doses of epinephrine, enlarged in stress, be able to adjust the fatty acid arrangement of phospholipids in the heart. In specific, an augment in AA and DHA amount, a reduction in LA, and a diminished ratio of n-6 to n-3, has been persuaded via catecholamines.

Hypolipidemic Effects:

The hypolipidemic effect of n-3 fatty acids is analogous to those of n-6 fatty acids, only if they restore saturated fats in the food. Advantage is shown by n-3 PUFA which in hypertriglyceridemic patients, constantly decreases serum triacylglycerol fractions, but the n-6 fatty acids do not with may constantly raise them (62). An additional significant deliberation is the discovery that throughout chronic fish-oil eating, post-prandial triacylglycerol levels reduces. Additionally,

Nestle (63) described to facilitate utilization of high amount of fish oil . Studies in human shows that fish oils decrease the degree of liver secretion of very low-density lipoprotein and triacylglycerol also in normolipidemic subjects, n-3 fatty acids avoid and quickly recover carbohydrate-induced hypertriglyceridemia.

Psoriasis:

In pathogenesis of cutaneous scaly disorders, AA metabolism plays a major role. In the lesions of patients with psoriasis elevated amounts of AA and its products LTB_4 and 12-hydroxyeicosatetraenoic acid (12-HETE) are found. Administration of i.v. n-3-fatty acid helps in decrease of psoriasis.

Ulcerative Colitis:

The products which are obtained from AA metabolism are LTB_4 and PGE_2, are amplified in patients having ulcerative colitis. LTB_4 is an vital intermediary of edema and has the capacity to enroll extra neutrophils from blood stream into mucosa, thus increases of LTB_4 (64).Intake of fish oil in patients having ulcerative colitis result in decrease in rectal dialysate levels of LTB4, weight increase and a fall in the dosage of prednisone (64).

Cancer:

Functions of fatty acids in humans having cancer has been insufficiently examined, elevated n-6 PUFA intake with respect to little ingestion of n-3 PUFA which increases danger of cancer in the breast, colon, and prostate. n-6 PUFA enhances tumor formation and its proliferation and migration while n-3 PUFA could constrain the progress of cancer cells.

Conclusions:

Dietary fatty acids are important for the majority of common diseases. To have information about health outcomes of dietetic PUFA, we should have improved perception of the importance of fatty acids in the human body. Most important aspects of PUFA physiologically are linked to relations with the nuclear receptor proteins. Interactions on eicosanoids metabolism are a new nutritional method to illnesses. Maintained by clinical confirmation, numerous establishments and administrations now distinguish and deliver investigation to discover in PUFA a novel curative approach to a extensive range of present ailments.

2.2 Omeprazole

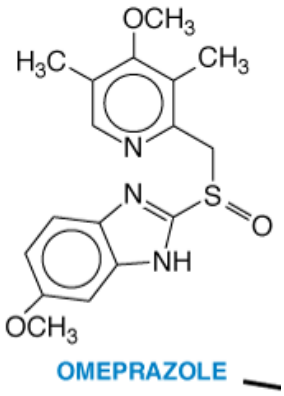


Figure 11: Structure of Omeprazole

Omeprazole is the new class of drugs, the acid pump inhibitors which control gastric acid secretion at the final stage of the acid secretory pathway and as a result reduce basal and stimulate acid secretion of the stimulus. Patients with gastric ulcers or duodenal , omeprazole as a single 20mg daily dose provide rapid with complete healing compare with ranitidine 150mg twice or 300mg nighttime or cimetidine

800 or 1000 mg/day. Patient's response to treatment is very poor with histamine Hs-receptor antagonists respond well to omeprazole - most ulcers healed in 4 to 8 weeks of omeprazole 40 mg/day therapy. Omeprazole 20 or 40 mg/day have been administered as protection therapy for peptic ulcer disease for up to 5.5 years by means of very few ulcer recurrences. In patients with ulcerative oesophagitis, omeprazole 20 or 40 mg/day produce healing in regarding 80% of patients following 4 weeks, as well as superior to ranitidine by respect to together healing and indication relief Healing rates of > 80% are achieve after 8 weeks in patients by severe reflux oesophagitis unresponsive to H_2-receptor antagonists.

To prevent relapse 20mg dose is given daily for about 12-month period as maintanence in 80% of patients. In Patients having Zollinger-Ellison syndrome, Omeprazole is consider to be most excellent pharmacological option for controlling gastric acid secretion. Daily dosage of 20 to 360 (median 60 to70mg effectively decrease basal acid production to goal level (< 10 mmol/h or < 5 mmol/h in patients by acute partial gastrectomy or esophagitis) throughout management for equal to 4 years.

Omeprazole is greatly tolerated in a brief study (up to 12 weeks); the report frequencies of severe side effect (about 1%) were alike to that observed in patients given a histamine H2- receptor blocker. The extensive acceptability of omeprazole has been examined in patients treated for 5.5 years. Mild hyperplasia, but no evidence of cell dysplasia, enterochromaffin-like (ECL) or neoplasia or ECL cell carcinoids has been accounted. Thus, omeprazole a extremely efficient alternate to other therapies accessible for reflux duodenal and gastric ulcers, oesophagitis,

together with those situation which are inadequately receptive to histamine H_2-receptor blockers. The capability of omeprazole as prophylaxis is required for peptic ulcer with reflux oesophagitis is assured and, wait for an confirmation of its extended period protection. Nonetheless omeprazole have attained a place of its expansion wherever it should obtain cautious concern by prescribe clinicians as a first-line drugs.

Pharmacological Properties:

Omeprazole manages acid emission by blocking of gastric K+, H+-ATPase, the enzyme which is accountable for the last step in the emission of hydrochloric acid by means of the gastric parietal cell. As a weak base, omeprazole concentrate in the acidic milieu of the secretory canaliculi wherever it is transformed by acid to its functioning sulphenamide by-product. In this type the treatment does not traverse membranes of the cells as well as is entrapped at its place of operation. Thus, omeprazole provide an effectual with precise way of balancing acid oozing in spite of the nature of the secretory stimulus, and blocks together basal with enthused gastric acid release. Gastrin let go from antral G cells is stimulated as gastric acid oozing is repressed, as a result, like other acid blockers, omeprazole would be present expected to raise the levels of plasma gastrin. Certainly, short term « 12 weeks) omeprazole treatment typically increase plasma gastrin level by means of 2- to 4-fold - 24-hour level following a 20mg daily dose are significantly less than those seen in patients with pernicious anaemia, similar to those achieve by parietal-cell vagotomy, and slightly superior than those seen with ranitidine 150mg twice daily. For the duration of longer term therapy, omeprazole 20 or 40 mg/day originally increase plasma gastrin levels, with no further raise noted for the duration

of extended therapy in most patients. Studies have shown to the alteration in gastric mucosal morphology observed in rats administered extremely high dose of omeprazole for prolonged period correspond to the effects of hypergastrinaemia going on in response to profound inhibition of acid secretion. Proliferation of ECL cell in the oxyntic mucosa along with the improvement of ECL-cell carcinoids have also be observed in rats with further antisecretory agents (including ranitidine) with follow partial corpectomy. The consensus analysis from available data is that the fair elevation in plasma gastrin level observed in clinical studies with therapeutic dose of omeprazole is unlikely to consequence in clinically important alteration in gastric morphology. Placebo-controlled study into healthy volunteers indicate so as to omeprazole 40mg daily otherwise further for greater than 4 days considerably reduce aspirin and naproxen-induced gastric mucosal injury, confirm earlier findings from animal studies. The bioavailability of omeprazole, administer as enteric-coated granules toward limit preabsorption acidic degradation, is concerning 65% in healthy volunteers. Peak plasma concentration and AUC value rise by repeated administration, signifying that absorption increases and/or first-pass hepatic metabolism become saturated. Omeprazole distributes rapidly and widely to extravascular sites V (=0.31 L/kg). Although the drug is rapidly eliminated from plasma (mean half-life 0.5 to I hour), its antisecretory effect persists for much longer since it is preferentially concentrated in parietal cells where it covalently binds to H+, K+- ATPase. Elimination is almost entirely by metabolism, follow by means of primarily urinary excretion. The major plasma metabolites are hydroxyomeprazole and omeprazole suiphone neither of which appears to be pharmacologically active. The disposition of omeprazole does not show to be changed in patients with renal disease, otherwise in those undergoing haemodialysis. Increased age with liver

disease delay plasma clearance of the drug other than this does not necessitate dosage modification in these patient groups.

Therapeutic Uses:

The original assessment of omeprazole recognized the role of the drug in the short term treatment of duodenal ulcer, as well as for reducing gastric acid hypersecretion in patients by Zollinger-Ellison syndrome, as well as demonstrated its potential in gastric ulcer with reflux oesophagitis. In the temporary many more clinical trials have been published. Omeprazole 20 mg/day provide a more quick response along with superior healing rates into patients with duodenal ulcer, along with faster relief of associated symptoms , than ranitidine 150mg twice daily or 300mg at night-time, or cimetidine 800 to 1000 mg/day. At these dosages, healing rates is 93% after 4 weeks compare to 83% in patients treat with ranitidine. In patients by gastric ulcer, omeprazole 20 to 40 mg/day be also more efficient than ranitidine 150mg twice daily or cimetidine 800 to 1000 mg/day, achieve healing rates of 73 and 91% after 4 and 8 weeks, compare by means of 62 and 85%, respectively, with ranitidine therapy in a meta-analysis of clinical studies. Omeprazole is a very effective in healing duodenal with gastric ulcers poorly receptive to histamine H_2-receptor antagonist treatment, by almost all patients showing complete healing within 4 to 8 weeks at a daily dose of 40mg. Relapse of healed duodenal ulcer following treatment by omeprazole or H2-receptor antagonists is inhibited is frequent, along with therefore maintenance therapy might be required. Weekend therapy (20mg administered daily for 3 days/week) appears to provide similar protection against relapse to a 10 mg daily dose; 6-month relapse rates range from 23 to 29% compare with > 60% in placebo-treated patients. Omeprazole 20 or

40 mg/day administer incessantly for up to 5.5 years in a small number of patients provide total protection next to ulcer relapse. Omeprazole 20, 40 and 60 mg/day is greater to placebo, ranitidine 300 mg/day as well as cimetidine 1600 mg/day in curative erosive and ulcerative lesions, with relieving symptoms in patients by reflux oesophagitis. After 4 weeks, curative rates were 81 and 6% in patients treat by omeprazole 20 or 40 mg/day and placebo, respectively, as well as 75 and 23% of patients be free of heartburn following 4 weeks. Relapse in patients by reflux oesophagitis occur former and more often than in patients with duodenal ulcer. Patients frequently involve long term treatment to prevent relapse: cumulative reduction rates following 12 months be 78 and 15% through incessant and weekend (3 days/week) omeprazole therapy (20mg daily), respectively. Corresponding rates for medium and high dose continuous histamine H_2-receptor therapy were 38 and 33%, respectively. In patients with Zollinger-Ellison syndrome a median omeprazole dosage of 60 to 70 rug/day reduces and maintains basal acid output at target levels « 10 mmol/h or < 5 mmol/h in patients with acute oesophagitis or partial gastrectomy with too quickly relieve acid-related symptom such while heartburn, abdominal pain as well as diarrhea throughout medication for up to 4 years.

Tolerability

In clinical trials Omeprazole is extremely tolerated in brief periods i.e. < 12 weeks. The occurrence of undesirable effects accounted in 19000 persons treated in a clinical study did not vary between omeprazole or placebo-treated patients, and in comparative studies, the incidence (1% of patients) and range of severe adverse effects was like to that connected with H_2-receptor blocker treatment. Gastrointestinal indications are the majority often accounted by means of patients

getting omeprazole or H_2- receptor blockers. Less than 2% of patients have ceased omeprazole handling because of bad actions in clinical trials, moreover there was no association connecting omeprazole dosage with occurrence of unfavorable effects. Through long term (up to 5.5 years) supervision of omeprazole at healing dosage, no ECL cell neoplasia or dysplasia have been seen.

Dosage & Administration:

A daily 20mg dose is suggested for the dealing with reflux oesophagitis, duodenal and gastric ulcer, though 40 mg/day may be essential in patients with circumstances poorly receptive to histamine H_2-receptor blocker treatment. reappearance of reflux oesophagitis has be effectively treated with daily 20 or 40mg dosage whilst a 10 mg daily dose appear capable in patients with ulcer in duodenal. Patients having Zollinger-Ellison syndrome, omeprazole 60 mg/day is optional at first with personal modification to continue target gastric acid production. Dose regulation is not essential in aged patients, or in patients by renal or hepatic impairment.

2.3 Ranitidine

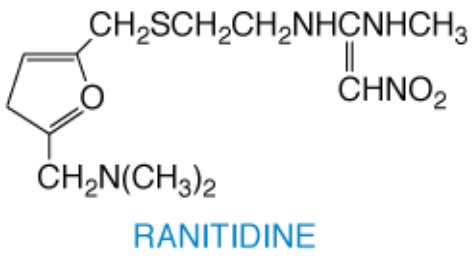


Figure 12: Structure of Ranitidine

Ranitidine is a histamine H_2receptor blocker that does not have an imidazole group. Comparing the degree of cure of gastric ulcers above a period of 4 to 6 weeks, ranitidine 150mg two times a day is an applicable alternate to 1000mg cimetidine every day. The incidence of ulcer recurrence is decreased by taking 150mg dose of ranitidine at night. Preliminary study in the Zollinger-Ellison disease with in patients who cannot tolerate, or insensitive to cimetidine, point to that ranitidine checks the gastric hyper-acidity which cures maximum ulcers, together with those which unsuccessful to respond to months of treatment with cimetidine 1 to 1.6g every day. In contrast to ranitidine, cimetidine has no androgen antagonistic effect and doesn't transform hepatic metabolism of drugs. Ranitidine is appropriately accepted. Initial articles on the cimetidine-induced negative effect subsequent replacement of ranitidine, recommend that ranitidine might be of worth in patients prejudiced of cimetidine. However, practice by ranitidine is desired to decide the medical significance of these articles.

3. AIMS AND OBJECTIVES OF THE STUDY

1) To examine and investigate the possible roles of polyunsaturated fatty acids (omega -3 & omega -6) and to study the anti-ulcerogenic effects in various models of induced ulcers in rat such as swimming induced stress ulcer, ethanol induced ulcer, pyloric ligation, indomethacin & histamine induced ulcer, reserpine induced ulcer, and dimaprit induced ulcer.

2) To investigate the effects of proton pump inhibitor, Omeprazole and H_2 receptor blocker like Ranitidine on different induced ulcer models.

3) To evaluate the effects of PUFA containing oils and combination of Proton pump inhibitor on gastric ulcer in different types of animal models.

4) Investigate the effects of PUFA containing oils and combination of H_2 receptor blocker induced effects on gastric ulcer models

5) To evaluate the effects of PUFA containing oils and conventional antiulcer drugs on biochemical parameters like the PGE2, iNOS activity, plasma TNF-α and IL-1β levels and superoxide dismutase activity in gastric mucosa.

4. SCOPE AND PLAN OF WORK

Several methods are there to evaluate the antiulcerogenic activity of the synthetic and natural products like aspirin induced ulcers, alcohol mediated ulcers, pyloric ligation mediated ulcers, indomethacin mediated ulcers, histamine mediated ulcer, reserpine mediated ulcers, serotonin induced ulcer, acetic acid induced ulcers, and Hydrochloric acid induced ulcers. Most of the researcher could evaluate the efficacy of various anti ulcer drugs by these methods.

There are a number of other experimental methods which are helpful in elucidating the various mechanisms like cytoprotection, H_2-antagonism, antisecretory, effect on gastric mucosal blood flow, gastric potential difference and involvement of biochemical alterations including gastric mucus, mucosubstances, prostacyclin, sulphydryls etc, which are involved in the ulcerogenic and/or gastroprotective effect of newer drugs. In spite of these advancements in the studies on the patho-physiology of peptic ulcer disease and introduction of highly effective H_2-blockers and gastric proton pump hydrogen potassium ATPase inhibitors, we have yet to discover an efficient anti-ulcer drug that not merely heals the peptic ulcers but also efficiently prevents their reappearance.

All *in vivo* animal models can be utilized to examine the defensive or healing functions of medication or drugs on the basis of the time of initiation of the peptic ulcer. For defensive studies, it is desirable to treat the animals for no less than two weeks previous to the ulcerogenic is introduced to provoke the peptic ulcer, following which the size of the scale of ulcers is noted with the suitable index to

decide the level of ulcer prevention attained. In the situation of restoring or curative models, the ulcers are stimulated following which the animals are treated for a minimum of two weeks and then the measurement of the size of ulcers with the suitable index to decide the scale of healing of the ulcers is done.

5. MATERIALS AND METHODS

5.1 CHEMICALS AND DRUGS:

Maxepa n-3 rich in fish oil (EPA & DHA) were obtain from Merck, India and from the Cayman chemical, USA Arachidonic acid n-6 were obtain and these were given as the supplements of PUFAs. Omeprazole (OMEZ) from Sigma was suspended in 1% of sodium Carboxy Methyl Cellulose (SCMC) and was given to the animals for ulcer protective study. The ulcer stimulating chemicals were given orally/subcutaneously, i/p or i/v. Indomethacin, histamine, Reserpine & Dimaprit (Sigma). For the superoxide dismutase activity the reagents and chemicals used were purchased from Sigma and Fisher chemicals. All procedures were approved and conceded out as per the rules of board for the reason of control and management of animal experiments (CPCSEA) # 3/243#, #10/243#, #28/243#.

5.2 EXPERIMENTAL ANIMALS & ETHICAL CONSIDERATION:

Albino Wistar rats of whichever sex of weight vary between 250-300 grams were used for the experiments. Animals were arbitrarily assigned to the treatment groups and was housed in polypropylene cages and preserved under standard circumstances (12 h light and dark cycles, at 25±3ºC and 35-60% humidity). All animals were permitted for free access to water and fed with standard commercial pelleted rat/mice chaw (M/s. Hindustan Lever Ltd, Mumbai). The study was approved by the Institutional Animal Ethical Committee, registered under CPCSEA, India. All the experimental methods and procedures utilized in this study were

evaluated by the Institutional Animal Ethics Committee and were in agreement with the rules of the IAEC.

5.3 PHARMACOLOGY STUDY:

5.4 VARIOUS METHODS OF INDUCING ULCERS IN RATS:

A) Ulcer induced by ethanol:

In experimental animals gastric lesions produced by ethanol (absolute) are reproducible methodology (65). In this experiment Wistar rats weight varying between 250 to 300 grams were used. Animals were fed with regular animal food. The animals were put on fast for 12 hours except water *ad libitum.* Orally, the animals were given, 0.1% tween 80. The absolute i.e. 99.9% ethanol was given to every rat in the concentration of 1 ml/200 gm body weight, after an hour of the administration of tween 80. After an hour with surplus of anesthetic ether the animal were euthanized and stomach was cut opened along the greater curvature, left over material was washed with normal saline and the inner surface was observed for ulcer wounds in the glandular region with the help of magnifying glasses and grade of severity of ulcer was ranked.

B) Swimming (stress) induced ulcer:

The method was established on previous available literature(66). Six groups of animals in each group divided into six (n=6). Ulcers were produced in various groups of animals by fasting them for 24 hours and then by making them to swim forcefully in the glass container of (height 45 cm, diameter 25 cm) holding water to

the height of 35 cm with the temperature of 25°C for 3 hours. One group served as control arm. The drug suspensions were made and delivered in the concentration of 0.2 ml/200g of body weight, 20 minutes prior to forced swimming test. The different groups were allocated as defined below.

C) Pyloric ligation method:

Fish oil or AA - rich oil or vehicle control or positive control drug was administered 20 minutes prior to pyloric ligation. Under the influence of anesthesia by ether, the abdomen was opened following which the pylorus was ligated. The abdomen was then sutured. After 4 hours of ligation, the animals were sacrificed with an excess dose of ether anesthesia for the dissection of stomach. Gastric juice was then gathered and its amount was calculated. The glandular section was next uncovered and inspected for ulceration and ulcer index was established(67)**.**

D) Indomethacin and Histamine induced ulcers:

Indomethacin is a NSAID which inhibit the synthesis of prostaglandins. Prostaglandins protect the gastric mucosa by producing leukotrienes and bicarbonate ions(68).

Procedure: Albino rats of either gender weighing between 250-300 gms are separated into five groups of six animals in each group. The animals are fasted for 24 hours. The test drug in varying concentrations based on the design of the experiment is administered at least 30 minutes before induction of ulcers.

Indomethacin (5 mg/kg) was first administered subcutaneously to rats fasted for 24 h, and then histamine dihydrochloride (40 mg/kg) was administered subcutaneously three times, at 2.5-h gap, starting 30 min after injection of indomethacin. This joint treatment induced one or two round lesions (9.8 ± 1.4 mm^2) in the proximal duodenum at a frequency of 100%, and a few lesions in the corpus and antrum of the stomach also. These methods signify that the growth of duodenal lesions stimulated by indomethacin plus histamine in rats is because of both an augmentation in gastric acid release and an impairment of acid-induced duodenal HCO_3^- release. This recently recognized model will be helpful for studying the pathogenesis of duodenal ulcers and for screening antiulcer of agents.

E) Reserpine induced ulcers:

Principle

Reserpine mediated gastric ulcers has been ascribed to the degranulation of gastric mast cells and resulting release of histamine which is supposed to be a cholinergically mediated (69).

Procedure

Adult albino rats weighing 250-300 gms were fasted for 24 hr. Animals were divided into different groups following water *ad libitum*. Reserpine (10mg/kg) administered intramuscularly rats. 30 minutes after the administration of the standard or test drug or control vehicle (Distilled water) intraperitoneally. All the

animals were sacrificed after 18 hr, their stomachs were taken out, opened all along the greater curvature and sum of lengths (mm) of all lesions for each rat was used as ulcer index and percentage protection of ulcers were calculated.

F) Dimaprit induced duodenal ulcer

Dimaprit, an H_2 receptor agonist, has been revealed to stimulate gastric erosions in rats after a single iv/ip dose. This model is particularly helpful for test of H_2 blockers(70).

Procedure:

Wistar rats (250 to 300g) are used for the experiment. The animals are fasted for 24 hours before the experiment but permitted unrestricted approach to water. In rats, dimaprit is given in a dose of 150mg/kg iv, single dose. The animal is sacrificed one hour later and the stomach dissected out and examined for gastric erosions. The test drug or vehicle is given orally 60 minutes before injecting dimaprit.

5.5 EXPERIMENTAL DESIGN:

The present experimental study design was carried out with the following models.

PROTOCOL: 1

A. Ethanol Induced Ulcer (Mucosal Damage):

Group – I : Rat received only 0.1% of Tween 80, served as control vehicle group.

Group – II : Rats received ethanol 1 ml/200 gm of 99.5 %, p.o., treated as ulcerated Control.

Group – III : Rats received omeprazole (20 mg/ kg) were the positive control,

Group – IV : Rats were given Ranitidine (30 mg/kg, p.o.+ EtOH

Group – V : Rats received fish oil 40µl/day/animal for 10 days+EtOH

Group – VI : Rats received AA-rich oil 40µl/day/animal 10 days+ EtOH

PROTOCOL: 2

B. Swim stress mediated gastric ulcer:

Group – I : Rat received only 0.1% Tween 80, served as vehicle control.

Group – II : Rats Swim stress (3 hours) treated as ulcerated control,

Group - III : Rats received omeprazole (20 mg/ kg) served as positive control,

Group – IV : Rats were given Ranitidine (30 mg/kg, p.o) + Swim stress

Group – V : Rats received fish oil 40µl/day/animal for 10 days+ Swim stress

Group – VI : Rats received AA-rich oil 40µl/day/animal 10 days+ Swim stress

PROTOCOL: 3

C. Pyloric ligation Method:

Group – I : Rat received only 0.1% Tween 80, served as vehicle control.

Group – II : Rats were given ranitidine (30 mg/ kg) served as positive control,

Group – III : Rats were given Ranitidine (30 mg/kg, p.o) + PL

Group –IV : Rats received fish oil 40µl/day/animal for 10 days+ PL

Group - V : Rats received AA-rich oil 40µl/day/animal 10 days+ PL

PROTOCOL: 4

D) Indomethacin and Histamine induced ulcers:

Group – I : Rats received only 0.1% Tween 80, served as vehicle control.

Group – II : Rats received Indomethacin 5 mg/kg s.c + Histamine 40mg/kg s.c, treated as ulcerated control,

Group – III : Rats were given omeprazole (20 mg/ kg) served as positive control,

Group – IV : Rats were given Ranitidine (30 mg/kg, p.o) + Indomethacin + Histamine

Group – V : Rats received fish oil 40µl/day/animal for 10 days+Indomethacin+ Histamine

Group – VI : Rats received AA-rich oil 40µl/day/animal 10 days+ Indomethacin + Histamine

PROTOCOL: 5

E) Reserpine induced ulcers:

Group – I : Rats received only 0.1% Tween 80, served as vehicle control.

Group – II : Rats were given Reserpine (10mg/kg) administered intramuscularly rats, treated as ulcerated control,

Group – III : Rats were given omeprazole (20 mg/ kg) served as positive control,

Group – IV : Rats received Ranitidine (30 mg/kg, p.o)+ Reserpine

Group – V : Rats received fish oil 40µl/day/animal for 10 days+ Reserpine

Group – VI : Rats were given AA-rich oil 40µl/day/animal 10 days+ Reserpine

PROTOCOL: 6

F) Dimaprit induced duodenal ulcer:

Group – I : Rats were given only 0.1% Tween 80, served as vehicle control.

Group – II : Rats received dimaprit is given in a dose of 150mg/kg IV, single dose administered, treated as ulcerated control,

Group – III : Rats were given omeprazole (20 mg/ kg) served as positive control,

Group – IV : Rats were given Ranitidine (30 mg/kg, p.o)+ dimaprit.

Group – V : Rats received fish oil 40µl/day/animal for 10 days+ dimaprit.

Group – VI : Rats received AA-rich oil 40µl/day/animal 10 days+ dimaprit.

5.6 DOSE SELECTION & DRUG TREATMENT:

The selection of dose for all drugs and pharmacological tools for the animal models of ulcer were based on the previous research work and preliminary studies conducted by us. The route of administration of almost all the drugs were given orally and intraperitoneal (i.p) or subcutanesouly (s.c) & i/p.

5.7 STATISTICAL METHODS:

The experimental figures were depicted as mean ± SEM. The statistical analysis was done by one way analysis of variance (ANOVA) followed by the post hoc analysis by Dunnett's test as well as Tukey-Kramer Multiple Comparisons Test using Graph pad Instat version 3. Statistically significant values were determined by 'P' value which is considered significant at the level of $P<0.05$ and highly significant at $P<0.001$.

5.8 STUDY PARAMETERS:

Estimation of parameters:

The estimation of the ulcer index was done based on previous methods (71).

5.6.1 ESTIMATION OF ULCER INDEX (UI):

The ulcer index was analyzed by intensity of gastric mucosal damage and is classified as below;

Score	Lesions
1.	1 mm or < 1
2.	1 to 2 mm
3.	Greater than 2mm

The UI was estimated by utilizing the standard formula: UI = 1 (No. of lesions of score 1) + 2 (No. of lesions of score 2) + 3(No. of lesions of score 3)/10

The whole count was divided by a factor of 10, which was designated as ulcer index.

$$\%\,\text{gastro protection} = \frac{(\text{UIC} - \text{UIT})}{\text{UIC x 100}}$$

Where,

UIC-Ulcer index of Control group,

UIT - Ulcer index of Test group

5.6.2 ESTIMATION OF FREE ACIDITY:

The amount of free acidity levels was analyzed according to the earlier explained formula (72).

1. Gastric juice content (1 ml) was collected in to a 100 ml conical flask, to this volume 2-3 drops of Topfer's reagent was mixed and titrated with 0.01 NaOH until all the of red colour vanishes and the colour of the solution turns to yellowish orange.

2. The quantity of alkali added was noticed. This quantity added corresponded to the free acidity.

3. Titration was done with 2-3 drops of phenolphthalein solution and was sustained till a distinct red color recurs.

4. The quantity of alkali added was also observed and noted down which matches to total acidity. Acidity was computed by means of the standard method:

   Acidity levels (mEq/Litre) = Volume of NaOH x Normality of NaOH x 100/0.1 gm

5.6.3 ESTIMATION OF SUPEROXIDE DISMUTASE ACTIVITY:

Activity of Superoxide dismutase was calculated on the basis of previously explained technique (73). To a volume of 0.25 ml of tissue homogenate, 0.75 ml of ethanol and 0.2 ml of ice cold chloroform were mixed and were later centrifuged. The supernatant was collected and used and to this 0.6 ml of 0.6 nM EDTA solution and 1.2 ml of buffer (0.1 M, pH 10.1) were added and mixed. The reaction was started by adding 0.5 ml of fresh adrenaline (1.8 nM) and the change in absorbance was determined at 480 nm. For blank, reaction mixture exclusive of the homogenate was utilized. The enzyme activity was designated as U/ml.

5.6.4 ESTIMATION OF MUCOSAL PGE_2 LEVELS:

Gastric mucosal frozen tissue (1 g) was added to 5.0 ml homogenize buffer (0.1 M phosphate (pH 7.4), containing 1mM of EDTA and 10µM of indomethacin and were homogenized. The lysis mixture was then centrifuged in a micro centrifuge tube at the speed 16,000 x rpm for 15min at 2°c to 8°C. The supernatant was then transferred to a new tube, and the total protein content was evaluated by using a protein assay method. PGE_2 levels were estimated utilizing a PGE_2 Kit (Abbexa Ltd).

5.6.5 MEASUREMENT OF THE INDUCIBLE NOS ACTIVITY:

Different specimens of the gastric mucosa tissue were homogenized in buffer having 10mM EDTA and centrifuged at a speed of 13,000 rpm at $4^{O}C$ for 5min. The supernatant was transferred to a new tube, and its total protein content of 10µg/µL. Gastric mucosal tissue inducible NOS activity was estimated with the NOS assay kit (Abbexa Ltd).

5.6.6 MEASUREMENT OF PLASMA TNF-A AND IL-1B LEVELS:

Samples of blood in EDTA- containing vials were spun at 1000 rpm for 10 min at $4^{o}c$. The levels of IL-1β and TNF-α were estimated by ELISA kits (Peprotech, USA) according to the instructions of the manufacturer (74).

5.6.7 DETERMINATION OF H+K+ATPASE ACTIVITY:

The H+K+ATPase enzyme activity was determined in ulcer animal models (75). The assay medium comprised of the 70 mM Tris buffer, pH 6.8, 5 mM $MgCl_2$ and the enzyme solution in the presence of 10 mM KCl and in a total volume of 1 ml and was later incubated for a duration of 1 h. The reaction was initiated by adding of 2mM ATP, and incubated at 37 ◦C for 20 minutes and the reaction was terminated by adding 10% TCA to the reaction mixture. After this centrifugation, 2.5 ml of ammonium molybdate and a volumed of 0.5 ml of l-amino-2-naphthol-4-sulphonic acid were added to the supernatant and the absorbance was read at 620 nm. Results are shown as mmol of Pi librated/min/mg protein.

6. RESULTS AND ANALYSIS

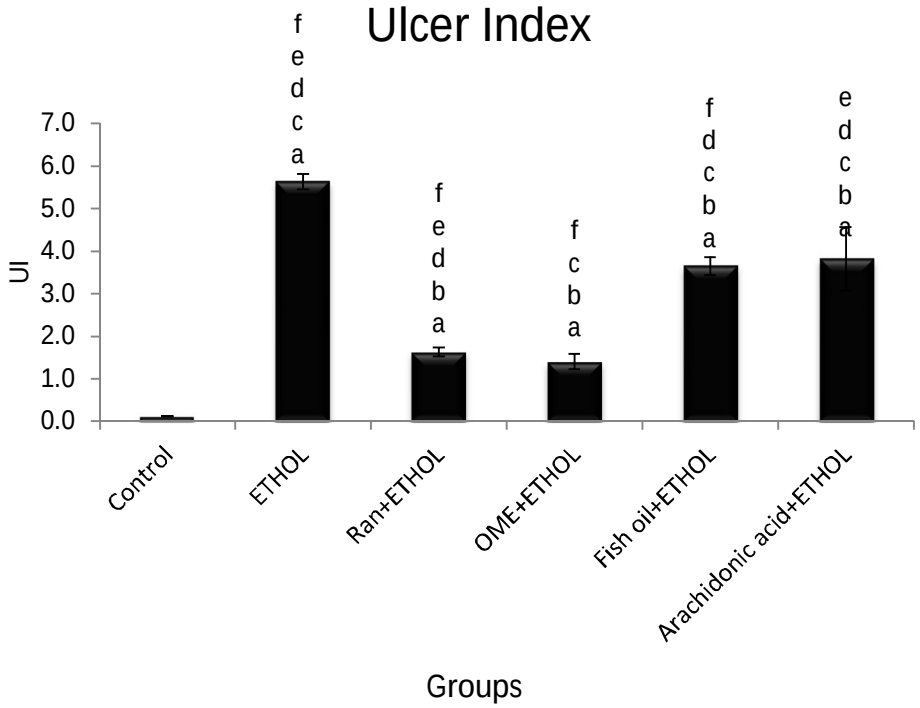
Gastric ulcer is one of the serious complications of gastrointestinal disorders. Various reasons account for it ranging from imbalance in the gastric pH to bacteria such as H. pylori (76- 77) categorize the management strategies into two regimens: (a) reducing the production of gastric acid and (b) rejuvenating the gastric mucosal layer. Present work is based on the hypothesis that fish oil and Arasco oil would improve the gastric ulcer condition.

6.1 ETHANOL INDUCED ULCER:

Ethanol was used to induce ulcer in experimental rats. Upon induction ethanol gave an ulcer index of 5.63 ± 0.18 which is in par with literature. Supporting our hypothesis, fish oil at a concentration of 40µl/day showed an ulcer index of 3.65 ± 0.21 offering a protection of 35.16%. On AA-rich oil administration post ethanol treatment, showed an ulcer index of 3.82 ±0.75 with 32.14% protection. We compared our results against Ranitidine and Omeprezole as standards, which showed ulcer indices of 1.63 ± 0.10 and 1.40 ± 0.18 with 71.04% and 75.13% respectively. The results indicate that fish oil and Arasco oil are protecting the stomach from ethanol mediated insults. When the efficiencies are compared standard compounds are better than FO and AA. But further optimization with different concentrations might improve the protection efficiencies. The data were analysed by one-way ANOVA followed by Dunnett's test.

Table 2: ***Effect of fish oil and Arasco oil on ulcer index and % gastro protection in ethanol induced gastric ulcer in rats (n=6)***

Group No.	Treatment (mg/kg)	Ulcer Index (Mean ± SEM)	%Gastro Protection
Gp- I	Ulcerated control (Ethanol 1ml/200gm, p.o.)	5.63 ± 0.18	-
Gp- II	Ranitidine (30mg/kg, p.o.), 20 min prior to SM	1.63 ± 0.10**	71.04**
Gp-III	Omeprazole (20mg/kg, p.o.) +ETHOL.	1.40 ± 0.18**	75.13**
Gp-IV	Fish oil (40µl/day, p.o.)+. ETHOL	3.65 ± 0.21*	35.16*
Gp- V	AA-Rich oil (40µl/day, p.o.) +ETHOL	3.82 ±0.75*	32.14



*Mean ± SEM (n=6), * p<0.05 and ** p<0.01 compared to ulcerated control group*

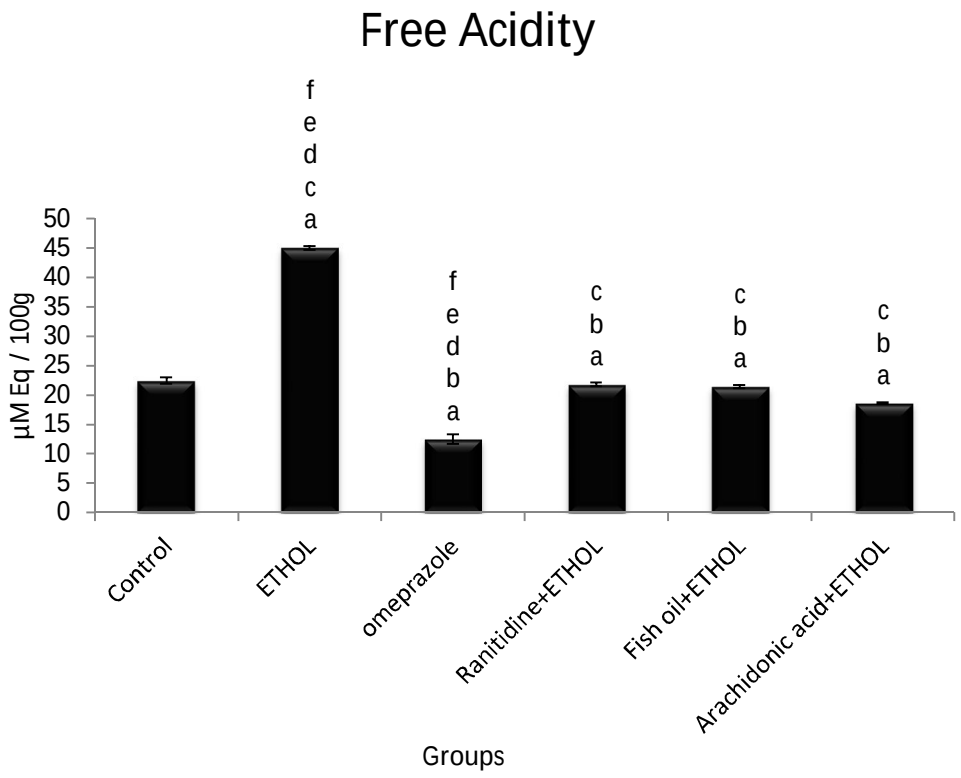
Figure 13: ***Effect of FO and AA on ulcer index and % gastro protection in ethanol induced gastric ulcer in rats. (n=6)***

Stomach free acidity:

Stomach free acidity is an index of gastric ulcer. It is expressed as the volume of alkali needed to neutralize the gastric fluid in the stomach. Free acidity in a control was found to be 22.43±0.566. In ethanol induced ulcer it was 45±0.339. The gastric content from fish oil and arachidonic acid treated animals required a volume of 21.4±0.258 and 18.6±0.179 respectively. On the other hand, Omeprazole and Ranitidine treatment showed a volume of 12.5±0.847 and 21.8±0.339 respectively. This experiment also showed the antiulcer potential of FO and AA. But the efficiency was clearly inferior to standard drugs due to the above mentioned reasons.

Table 3: Effect of fish oil and Arasco oil on free acidity in ethanol induced gastric ulcer in rats (n=6)

group No.	**Treatment (mg/kg)**	**Free acidity Mean ± SEM**
Gp –I	Control	22.43±0.566
Gp-II	Ulcerated control (Ethanol 1ml/200gm, p.o.)	45±0.339 ^{acdef}
Gp-III	Omeprazole 20mg/kg	12.5±0.847^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Ethanol	21.8±0.339^{abc}
Gp-V	Fish oil (40µl/day.p.o) + ETHOL	21.4±0.258^{abc}
Gp-VI	Arachidonic acid (40µl/day.p.o) +ETHOL	18.6±0.179^{abc}



Values are expressed as Mean ± SEM of six animals. The statistical significance of the data is p<0.05. a-Controlvs. others; b-ETHOL vs. others; c-Omeprazole vs others, Ranitidine+ETHOL vs others; e-Fish oil+ETHOL vs others; f -AA+ETHOL

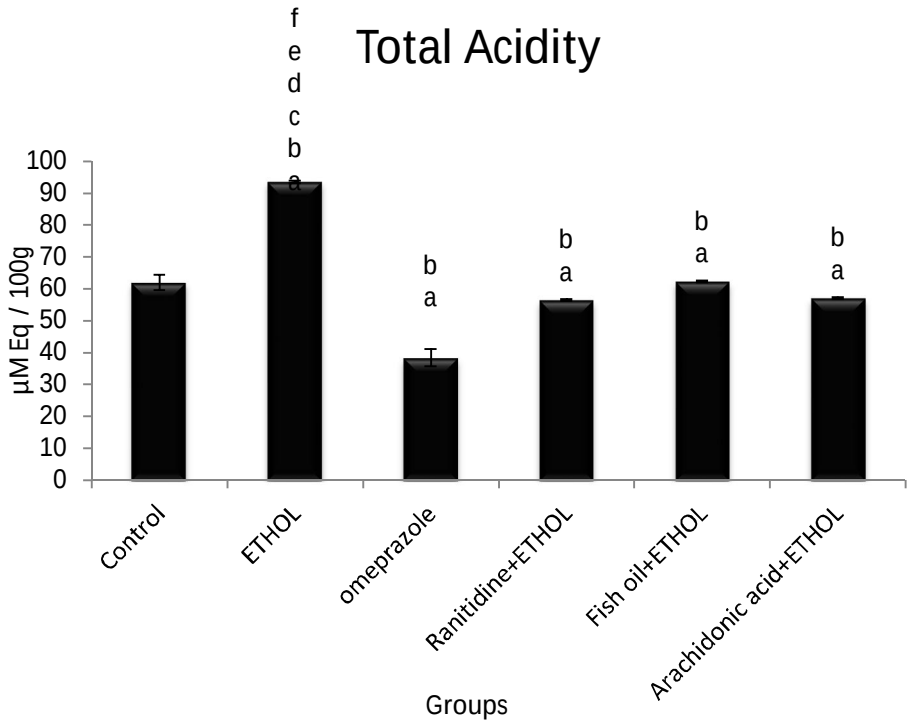
Figure 14: Effect of FO and AA on free acidity in ethanol induced gastric ulcer (n=6)

Stomach total acidity:

In control group, total acidity was found to be neutralized by a volume of 62 ±2.394 alkali, whereas, in ethanol induced ulcer model it was 93.5 ±0.402. Fish oil and Arasco oil controlled the total acidity with volumes of 62.33 ±0.284 and 57.06 ±0.327 respectively. Standard compounds, Omeprazole and Ranitidine showed values of 38.33 ±2.716 and 56.5 ±0.286 respectively.

Table 4: Effect of fish oil and Arasco oil on total acidity in ethanol induced gastric ulcer in rats (n=6)

group No.	Treatment (mg/kg)	Total acidity Mean ± SEM
Gp –I	Control	62 ±2.394
Gp-II	Ulcerated control (Ethanol 1ml/200gm, p.o.)	93.5 ±0.402^{bcdef}
Gp-III	Omeprazole 20mg/kg	38.33 ±2.716^{ab}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Ethanol	56.5 ±0.286^{ab}
Gp-V	Fish oil (40µl/day.p.o) + ETHOL	62.33 ±0.284^{ab}
Gp-VI	Arachidonic acid (40µl/day.p.o) + ETHOL	57.06 ±0.327^{ab}



Values are expressed as the mean ± SEM (n=6). The S S of the data is p<0.05. A-Control vs. others; b-ETHOL vs. others; c-Omeprazole vs others, Ranitidine+ETHOL vs others; e-Fish oil+ETHOL vs others; f -AA+ETHOL

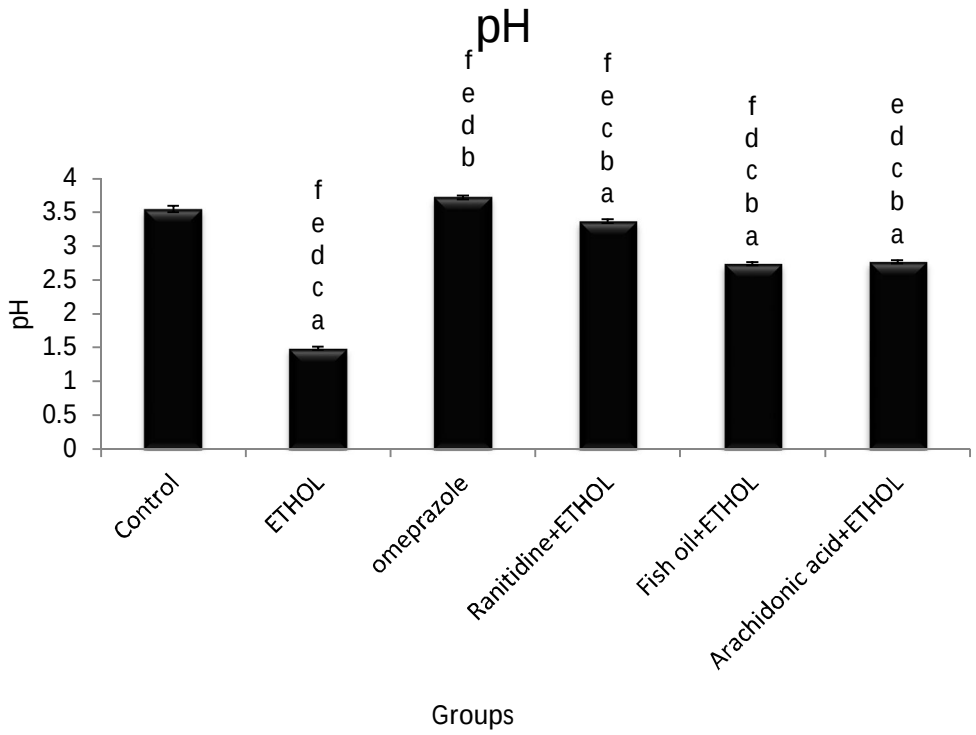
Figure 15: Effect of fish oil and Arasco oil on total acidity in ETHOL (n=6)

Gastric pH:

pH is the direct indication of acidity of stomach. The pH of control animal's stomach was found to be 3.550±0.049. When ulcer was induced with ethanol the acidity of the stomach reached a pH as low as 1.490±0.022. Fish oil and Arachidonic acid treatment increased the stomach pH to 2.740±0.027 and 2.77±0.024 respectively. Among the standard compounds Omeprazole was efficient in increasing the pH to 3.723±0.030 while Ranitidine could marginally increase the pH to 3.370±0.027. Overall, fish oil and arachidonic acid treatments significantly reduced the pH of the stomach content.

Table 5: Effect of fish oil and Arasco oil on pH in ETHOL (n=6)

group No.	**Treatment (mg/kg)**	**pH Mean ± SEM**
Gp –I	Control	3.550±0.049
Gp-II	Ulcerated control (Ethanol 1ml/200gm, p.o.)	1.490 ± 0.022^{acdef}
Gp-III	Omeprazole 20mg/kg	3.723 ± 0.030^{bdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Ethanol	3.370 ± 0.027^{abcef}
Gp-V	Fish oil (40µl/day.p.o) + ETHOL	2.740 ± 0.027^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) + ETHOL	2.77 ± 0.024^{abcde}



Values are expressed as mean ± SEM (n=6). The SS of the data is p<0.05. a-Control vs. others; b-ETHOL vs. others; c-Omeprazole vs others, Ranitidine+ETHOL vs others; e-Fish oil+ETHOL vs others; f -AA+ETHOL

Figure 16: Effect of FO and AA on pH in ETHOL (n=6)

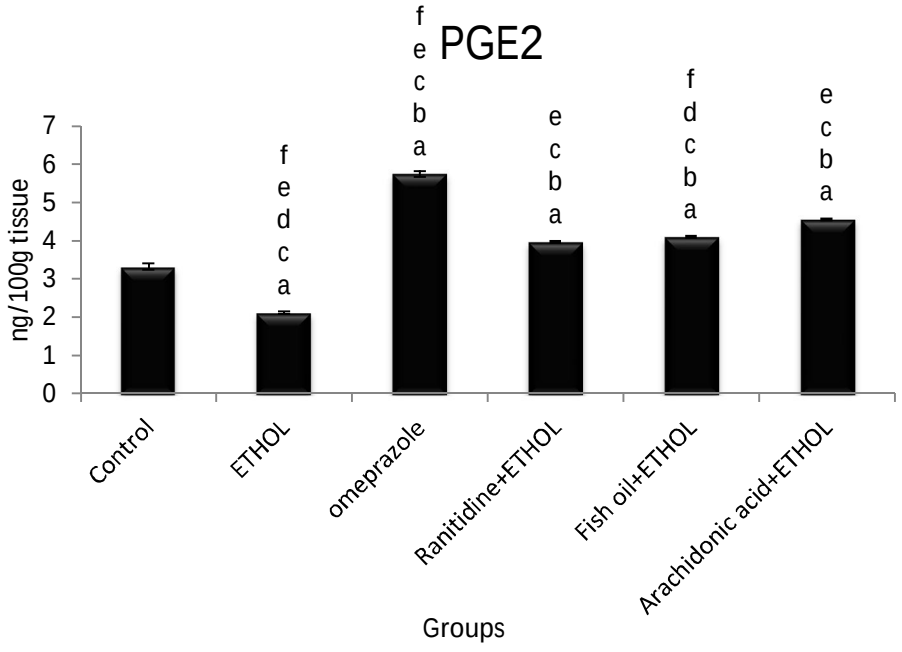
Expression of Prostaglandin E2:

Prostaglandin E_2 plays a vital role in wound healing (78- 79) has shown that PGE_2 helps in gastric ulcer healing by EP_4 receptors and results in VEGF expression(79). In our experiments also, we observed a reduction in PGE_2 levels during ethanol induced ulcer formation. Ethanol induced ulcer reduced the expression of PGE_2 to 2.12 ±0.028 ng/100g of tissue from 3.325 ±0.077ng/100g of tissue in control animals. Fish oil and Arachidonic acid increased the expression of PGE_2 to 4.1 ±0.026ng/100g and 4.56 ±0.023 ng/100g respectively. These values are much higher compared to the standard compound Ranitidine with ethanol induced ulcer background, which is marginally lower to fish oil with 3.97 ±0.027 ng/100g. Administration of standard compound Omeprazole without induction of ulcer

elevated the expression of PGE_2 to as high as 5.755 ±0.075ng/100g. These results indicate that fish oil and arachidonic acid are better than standard compound Ranitidine in promoting the expression of PGE_2.

Table 6: ***Effect of fish oil and Arasco oil on PGE_2 in ethanol induced gastric ulcer in rats (n=6)***

group No.	Treatment (mg/kg)	PGE2 Mean ± SEM
Gp –I	Control	3.325 ±0.077
Gp-II	Ulcerated control (Ethanol 1ml/200gm, p.o.)	2.12 ±0.028^{acdef}
Gp-III	Omeprazole 20mg/kg	5.755 ±0.075^{bcef}
Gp-IV	Ranitidine (30mg/kg.p.o) prior to Ethanol	3.97 ±0.027^{abce}
Gp-V	Fish oil (40µl/day.p.o) + ETHOL	4.1 ±0.026^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) + ETHOL	4.56 ±0.023^{abc}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Control vs. others; b-ETHOL vs. others; c-Omeprazole vs others, Ranitidine+ETHOL vs others; e-Fish oil+ETHOL vs others; f -AA+ETHOL

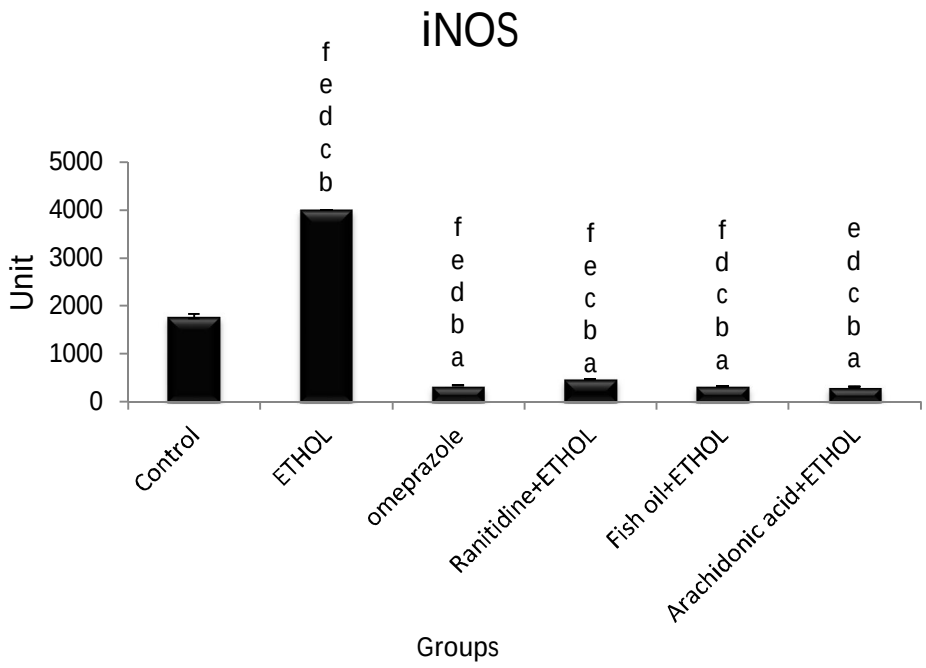
Figure 17: ***Outcome of fish oil as well as Arasco oil on PGE_2 in gastric ulcer stimulated by ethanol in rats(n=6)***

Expression of iNOS:

Disturbances in the balance of constitutive NO synthase (cNOS) and inducible NO synthase (iNOS) is one of the contributing factors in the development of gastric mucosal lesions. During the progression of the condition cNOS level decreases paralleling an increase in iNOS (80- 81) inducible NO synthase (iNOS) expression drastically increased from 1784.333±52.509 units (control) to 4004±1.073 units in ethanol induced ulcer model animal. Both fish oil and Arachidonic acid treatments following ethanol induced ulcer formation reduced the expression of iNOS expression to about 338.11±0.354 and 314.42±0.311 units respectively. Ranitidine treatment post ethanol treatment reduced the iNOS expression to 485.3±2.164 units. Omeprazole, ulcer stress could reduce the expression of iNOS to about 339.5±5.303 units. In conclusion, fish oil and arachidonic acid could effectively control the expression of iNOS and their efficacies are better than standard drug Ranitidine.

Table 7: Effect of fish oil and Arasco oil on iNOS in ethanol induced gastric ulcer in rats (n=6)

group No.	**Treatment (mg/kg)**	**iNOS Mean ± SEM**
Gp –I	Control	1784.333±52.509
Gp-II	Ulcerated control (Ethanol 1ml/200gm, p.o.)	4004 ± 1.073^{bcdef}
Gp-III	Omeprazole 20mg/kg	339.5 ± 5.303^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o) prior to Ethanol	485.3 ± 2.164^{abcef}
Gp-V	Fish oil (40µl/day.p.o) + ETHOL	338.11 ± 0.354^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) + ETHOL	314.42 ± 0.311^{abcde}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Control vs. others; b-ETHOL vs. others; c-Omeprazole vs others, Ranitidine+ETHOL vs others; e-Fish oil+ETHOL vs others; f -AA+ETHOL

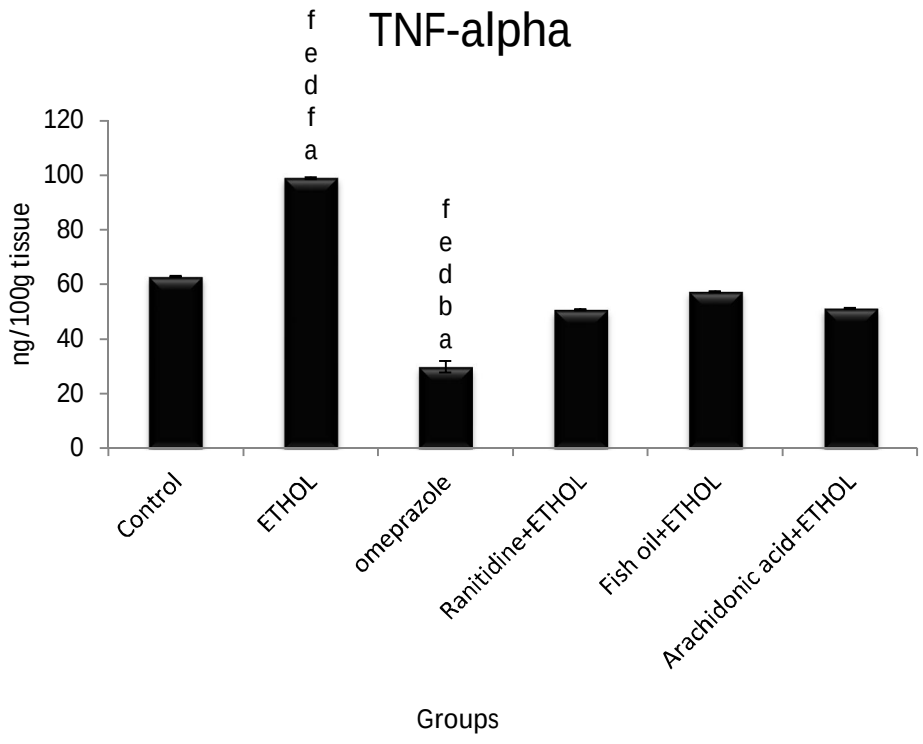
Figure 18: Outcome of fish oil and Arasco oil on iNOS in ETHOL (n=6)

Expression of TNF-α:

Tumor necrosis factor-α is one of the cell signaling factors having a major role in progression of inflammation produced mainly by macrophages. It has been shown to promote leukocyte adhesion during gastric damage and thereby promoting inflammation(82) Control animals showed an expression level of 63±.217ng/100g of TNF-α. The TNF-α level rose to 99±.349 after an insult of ethanol exposure. Fish oil and Arachidonic acid reduced it to 57±.217 and 51±.280ng/100g of tissue respectively. These values are much comparable to the standard drug Ranitidine to a level of 51±.266ng/100g of tissue. Similarly, on an untreated control Omeprazole reduced the level of TNF-α to 30±2.197. Therefore, it is evident that fish oil and arachidonic acid both are efficient in reducing the TNF-α to level as good as a standard drug could.

Table 8: ***Effect of fish oil and Arasco oil on TNF-alpha in ethanol induced gastric ulcer in rats (n=6)***

group No.	Treatment (mg/kg)	TNF-alpha Mean ± SEM
Gp –I	Control	63±.217
Gp-II	Ulcerated control (Ethanol 1ml/200gm, p.o.)	99±.349
Gp-III	Omeprazole 20mg/kg	30±2.197
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Ethanol	51±.266
Gp-V	Fish oil (40µl/day.p.o) + Ethanol	57±.217
Gp-VI	Arachidonic acid (40µl/day.p.o) + Ethanol	51±.280



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Control vs. others; b-ETHOL vs. others; c-Omeprazole vs others, Ranitidine+ETHOL vs others; e-Fish oil+ETHOL vs others; f -AA+ETHOL

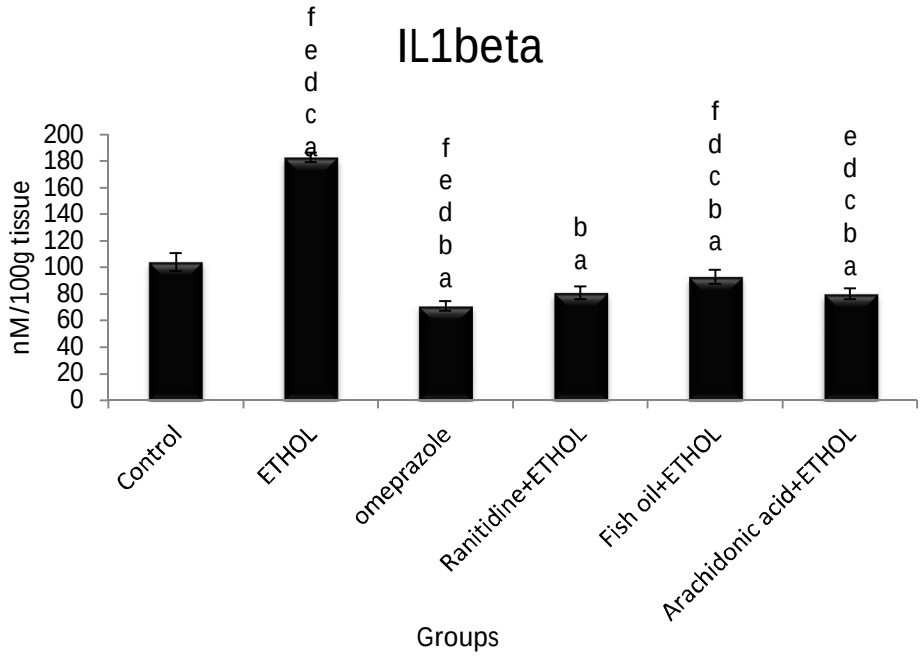
Figure 19: ***Outcome of fish oil and Arasco oil on TNF-alpha in gastric ulcers produced by ethanol in rats (n=6)***

Expression of IL-1β:

Inflammation of gastric mucosal epithelium is associated with TNF-α expression but also with a variety of other cytokines. One amongst them is IL-1. IL-1 β has multiple roles in the gastrointestinal tract including cytoprotective, anti-secretory and promotes PGE_2 synthesis, and slows-down the gastric emptying. (83). Gastric acid secretion and subsequent ulcer formation has been shown to be subsided by administration of IL-1α. (84) On the other hand, IL-1β exerts its action by playing at hypothalamic sites thereby reducing the gastric secretion in rodent model. (85) Therefore we estimated the levels of IL-1β. In control the level of IL-1β was around 104±6.730nM/100g tissue which on stress with ethanol administration rose to a level of 183±3.596 nM/100g tissue. Fish oil and arachidonic acid reduced it to 93±5.170 and 80±3.945 nM/100g tissue respectively. Standard drug Ranitidine lowered the IL-1β level to 81±4.833 nM/100g tissue. Omeprazole without ethanol administration reduced IL-1β level to about 71±3.697 nM/100g tissue. Overall, it is apparent that fish oil and arachidonic acid lowered the level of IL-1β to levels comparable to standard drug Ranitidine.

Table 9: Effect of fish oil and Arasco oil on IL1-betain ethanol (n=6)

group No.	**Treatment (mg/kg)**	**IL1-beta Mean ± SEM**
Gp –I	Control	104±6.730
Gp-II	Ulcerated control (Ethanol 1ml/200gm, p.o.)	$183±3.596^{acdef}$
Gp-III	Omeprazole 20mg/kg	$71±3.697^{abdef}$
Gp-IV	Ranitidine (30mg/kg.p.o) prior to Ethanol	$81±4.833^{ab}$
Gp-V	Fish oil (40µl/day.p.o) + Ethanol	$93±5.170^{abcdf}$
Gp-VI	Arachidonic acid (40µl/day.p.o) + Ethanol	$80±3.945^{abcde}$



Each bar represents the mean ± SEM of six animals. The statistical significance of the data is p<0.05. a-Control vs. others; b-ETHOL vs. others; c-Omeprazole vs others, Ranitidine+ETHOL vs others; e-Fish oil+ETHOL vs others; f -AA+ETHOL

Figure 20: Role of fish oil and Arasco oil on IL1-beta in ethanol (n=6)

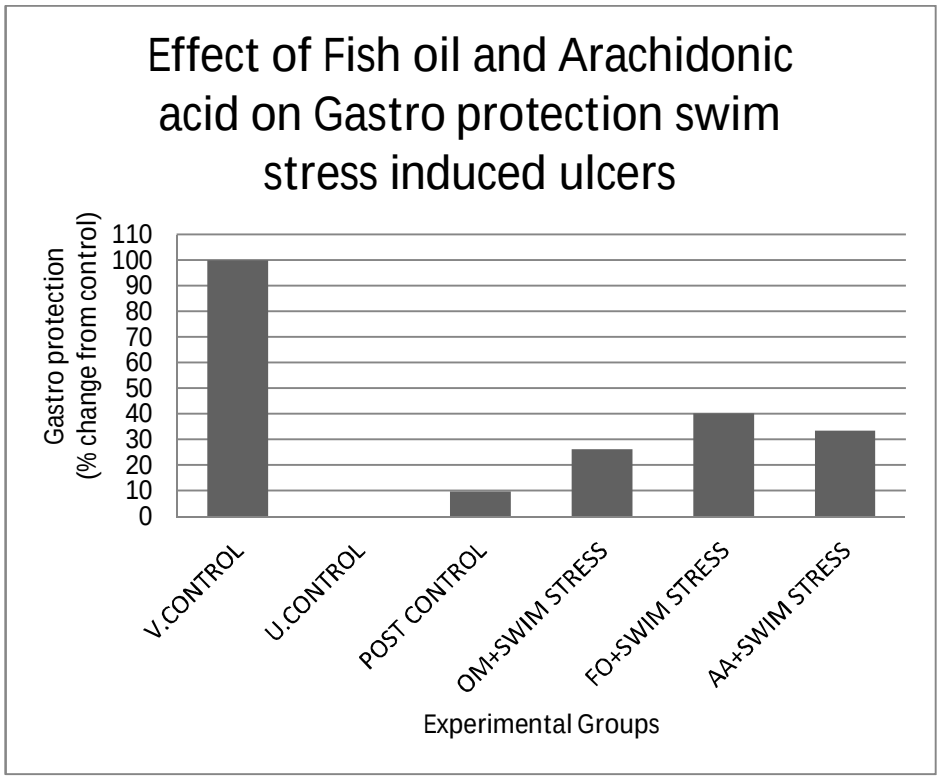
6.2 FO AND AA ON (SWIMMING) STRESS

The ulcerated controls give an ulcer index of 4.61 ± 0.18 while fish oil prevented the ulcer index of 3.65 ± 0.21 which is significantly lower than the ulcerated control. Similarly, arachidonic acid prevents ulcer formation with an ulcer index of 3.47 ± 0.75. The standard drug Ranitidine, gave an ulcer index of 3.2 ± 0.10 which is much lower than the ulcerated control. Omeprazole on the other hand lowered the ulcer index 2.63 ± 0.18.

The protection percentage of fish oil was 20.82% and arachidonic acid rich oil gave a protection of 24.72 while the standard compound Ranitidine protected from gastric ulcer to an extent of 30.58%. Omeprazole in a background of untreated control gave a protection percentage of about 42.95%.

Table 10: Effect of FO and AA on UI and % (swimming) stress (n=6)

Group No.	Treatment (mg/kg)	Ulcer Index (Mean ± SEM)	%Gastro Protection
Gp- I	Ulcerated control (Swim stress Control)	4.61 ± 0.18	-
Gp- II	Ranitidine(30mg/kg,p.o.) 20 mins before to SM	3.2 ± 0.10**	30.58*
Gp- III	Omeprazole(20mg/kg, p.o.) 20 mins before to SM	2.63 ± 0.18	42.95*
Gp- IV	Fish oil (40µl/day, p.o.) 20 mins before to SM	3.65 ± 0.21**	20.82
Gp- V	AA-Rich oil (40µl/day, p.o.) 20 mins before to SM	3.47 ± 0.75**	24.72



*Values are expressed as mean ± SEM (n=6), * p<0.05 compared to ulcerated control group.*

***Figure 21: Effect of fish oil and Arachidonic acid (PUFA) on % (swimming) stress in rats* (n=6)**

6.3 PYLORUS LIGATION

Introduction

Pylorus ligation is one of the very old methods to induce gastric ulcer. It is the choice of a number of research groups for its simplicity and reproducibility (86), (87). Therefore, we performed the assays to evaluate the efficacy of fish oil and Arasco oil to ameliorate gastric ulcer induced by pylorus ligation. Moreover, the assays since already performed in ethanol induced ulcer model would provide much insight into the efficiency of FO and AA.

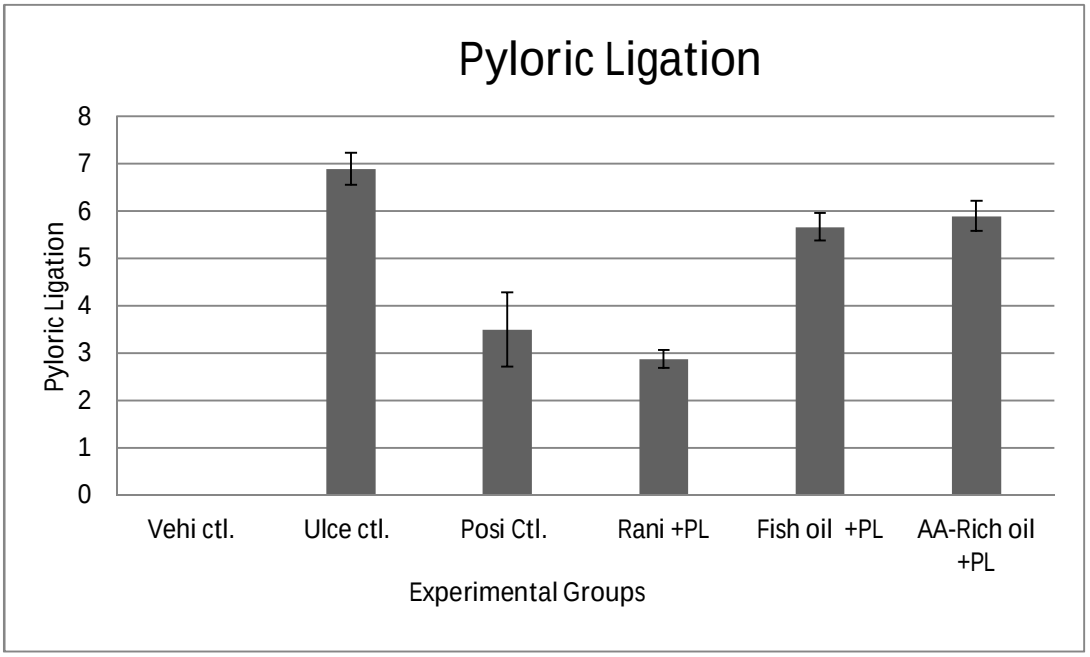
Effect of FO and AA on Pylorus ligation induced ulcer

Ulcer index is the direct measure of the extent of ulcer induced by a given inducing agent (88). In the current experiment, the ulcer was made using pylorus ligation method (86). The mean ulcer index in animals with pylorus ligation was 6.9±0.34 whereas, fish oil treated animals showed an ulcer index of 5.67 ± 0.29 providing evidence that fish oil has some protective action on ulcer. Interestingly, Arasco oil also provided some protection against pylorus ligation induced ulcer with an ulcer index of around 5.9 ± 0.32. The standard drug, Ranitidine could efficiently control the ulcer with an index of 2.88 ± 0.19 with a protection to an extent of 58.26%. On the other hand, Omeprazole provided an ulcer index of 3.5 ± 0.78The ulcer indices induced by two different techniques (ethanol and pylorus ligation) did not vary significantly. But the protection offered by two different oils, fish oil and Arasco oil varied to a greater extent. Fish oil could protect efficiently against ethanol induced ulcer (ulcer index 3.65 ± 0.21) than pylorus ligation (ulcer index 5.67 ±

0.29). And the same pattern was seen with Arasco oil indicating that the effectiveness of the oil depends on the mode of ulcer induction.

Table 11: Effect of FO and AA on ulcer index in pylorus ligated rats (n=6)

Group No.	**Treatment (mg/kg)**	**Ulcer Index (Mean ± SEM)**	**% Gastro Protection**
Gp- I	Ulcerated control (pyloric ligation)	6.9±0.34	-
Gp- II	Omeprazole (20mg/kg, p.o.)	3.5 ± 0.78	49.27
Gp- III	Ranitidine (30mg/kg, p.o.), followed by pyloric ligation.	2.88 ± 0.19	58.26
Gp- IV	Fish oil (40µl/day, p.o.) + pyloric ligation.	5.67 ± 0.29	17.82
Gp- V	AA (40µl/day, p.o.) + pyloric ligation.	5.9 ± 0.32	14.49



*Mean ± SEM (n=6),*p<0.05 and**p<0.01*

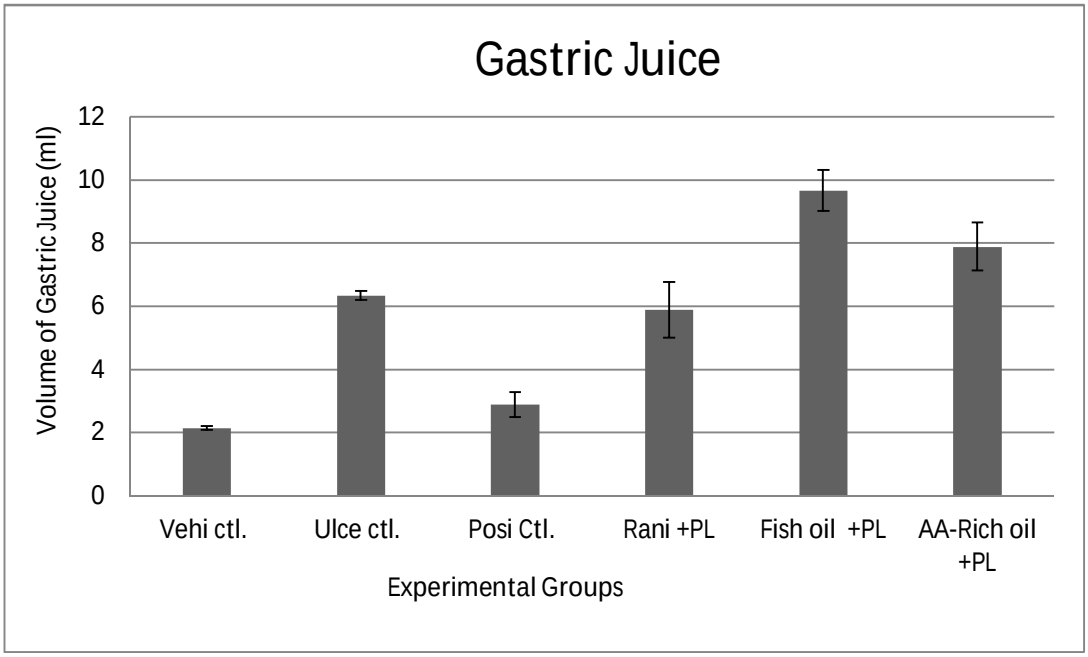
Figure 22: Effect of fish oil and Arasco oil on ulcer index in pylorus ligated rats (n=6)

FO and AA on gastric juice content

We measured the volume of gastric juice which has direct effect on ulcer induction. The vehicle contained a volume of 2.15±0.06 whereas the ulcerated control showed an index of 3.34+0.14. Animals are treated FO and AA treated a had a gastric content of 4.39 ± 0.03 and 3.96 ± 0.03 respectively. Ranitidine, the standard drug, showed a gastric volume of 3.35 ± 0.040. On the other hand, Omeprazole, in untreated control animals induced a volume of 2.89 ± 0.39. Fish oil and Arasco oil did not effectively reduce the volume of gastric juice. But they could effectively control the gastric ulcer. Overall, these results suggest that the protective effect of the given oils could be attributable to an increase in volume of gastric juice.

Table 12: Effect of FO and AA on volume of gastric content in pylorus ligated rats (n=6)

Group No.	**Treatment (mg/kg)**	**Volume (Mean ± SEM)**
Gp- I	Vehicle	2.15±0.06
Gp-II	Ulcerated control (pyloric ligation)	3.34+0.14
Gp-III	Omeprazole (20mg/kg, p.o.)	2.89 ± 0.39
Gp-IV	Ranitidine (30mg/kg, p.o.), followed by pyloric ligation.	3.35 ± 0.040
Gp- V	Fish oil (40µl/day, p.o.) followed by pyloric ligation.	4.39 ± 0.03
Gp-VI	AA-Rich oil (40µl/day, p.o.) followed by pyloric ligation.	3.96 ± 0.03



*Mean + SEM (n=6),*p<0.05 and**p<0.01*

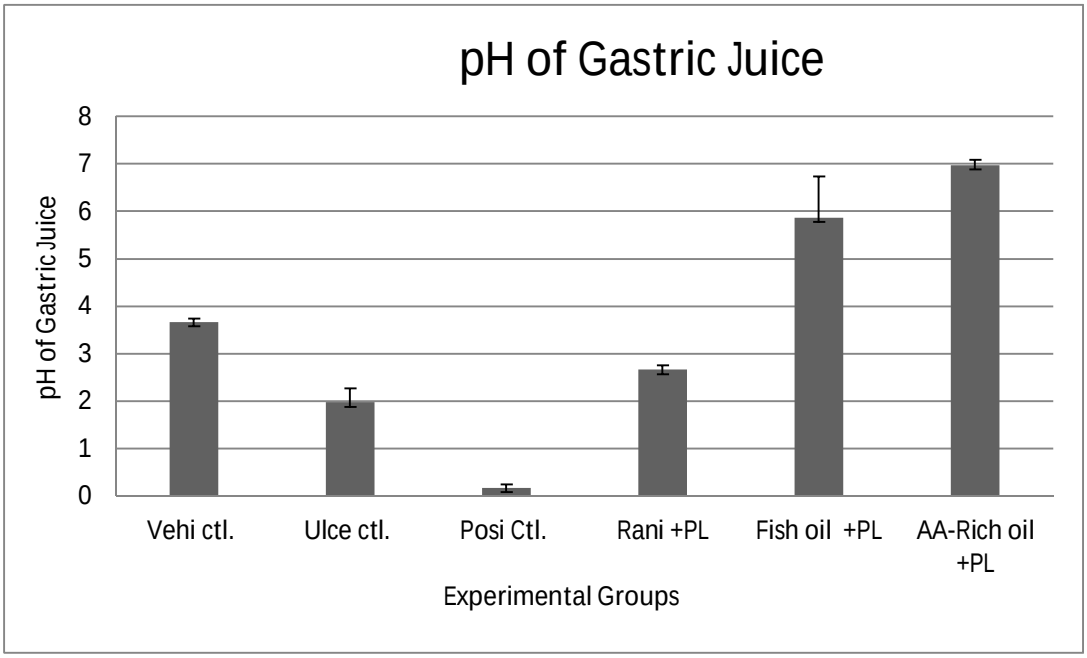
Figure 23: FO and AA on volume of gastric content in pylorus ligated rats (n=6)

FO and AA on pH

The stomach is extensively exposed to extreme acidic pH range which could play roles in ulcer formation and its protections. Therefore, we measured the pH of the stomach in various groups. Untreated controls showed a pH of 3.67±0.07 while in the ulcerated controls the pH was at an extreme of 1.98±0.28. Treatment with fish oil neutralized the pH and brought the pH to 5.87 ± 0.87. Most interestingly, Arachidonic acid brought the stomach pH almost near to neutral with a value 6.98 ± 0.1. The standard drug Ranitidine altered the pH to 2.67 ± 0.08 and Omeprazole in untreated control turned the pH to 0.18 ± 0.06 making it much more acidic. The experimental results from pH of stomachs revealed that the protective effect of fish oil and Arasco oil could be attributed to neutralizing ability of the oils.

Table 13: FO and AA on pH (n=6)

Group No.	Treatment (mg/kg)	pH (Mean ± SEM)
Gp- I	Vehicle	3.67±0.07
Gp-II	Ulcerated control (pyloric ligation)	1.98±0.28
Gp-III	Omeprazole (20mg/kg, p.o.)	0.18 ± 0.06
Gp-IV	Ranitidine (30mg/kg, p.o.), followed by pyloric ligation.	2.67 ± 0.08
Gp- V	Fish oil (40µl/day, p.o.) followed by pyloric ligation.	5.87 ± 0.87
Gp- VI	AA (40µl/day, p.o.) + pyloric ligation.	6.98 ± 0.1



*Mean ± SEM (n=6), * p<0.05 and ** p<0.01 In comparison to control group with ulcers*

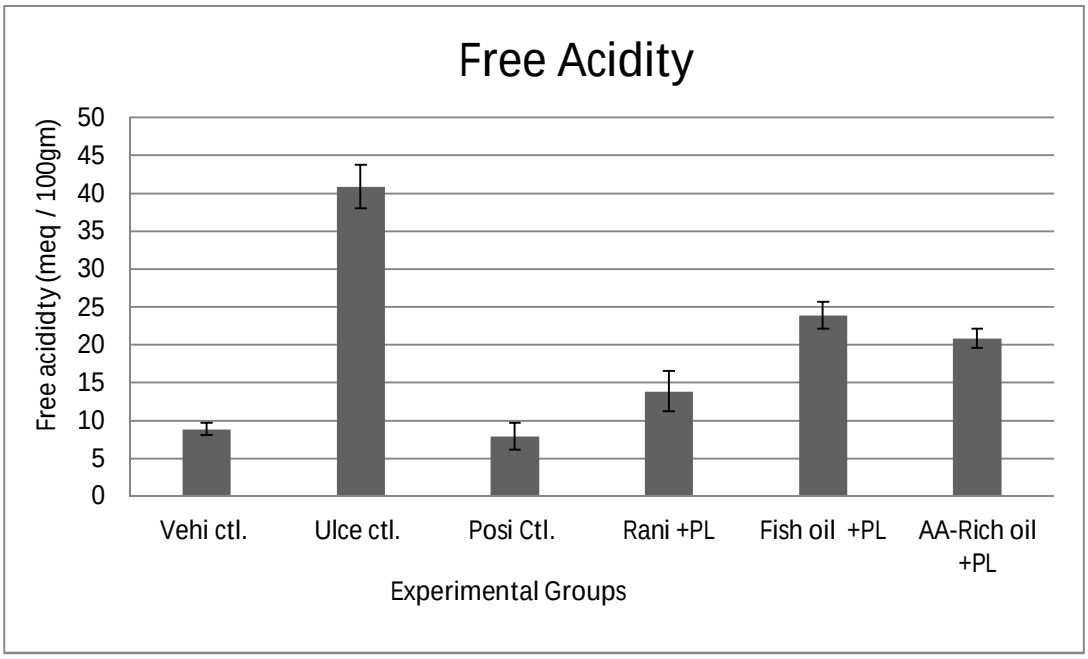
Figure 24: FO and AA on pH in pylorus ligated rats.

FO and AA on free acidity

The untreated controls had a free acidity value of 8.89±0.8 while the ulcerated controls showed a value of 40.89±2.87. Fish oil reduced the free acidity near to half of its original value ie. 23.89 ± 1.76 Similarly, Arasco oil reduced the free acidity as low as 20.85 ± 1.27 Ranitidine, the standard drug, showed a value of around 13.89 ± 2.7 showing a much reduction in neutralizing ability with respect to free acidity. Omeprazole, in untreated animals, on contrary, reduced the free acidity still lesser than the control.

Table 14: FO and AA on free acidity in pylorus ligated rats (n=6)

Group No.	**Treatment (mg/kg)**	**Free acidity (Mean ± SEM)**
Gp- I	Vehicle	8.89±0.8
Gp-II	Ulcerated control (pyloric ligation)	40.89±2.87
Gp- III	Omeprazole (20mg/kg, p.o.)	7.9 ± 1.78
Gp- IV	Ranitidine (30mg/kg, p.o.), followed by pyloric ligation.	13.89 ± 2.7
Gp- V	Fish oil (40µl/day, p.o.) followed by pyloric ligation.	23.89 ± 1.76
Gp- VI	AA (40µl/day,p.o.) +pyloric ligation.	20.85 ± 1.27



*Mean ± SEM (n=6), * p<0.05 and ** p<0.01 compared to ulcerated control group*

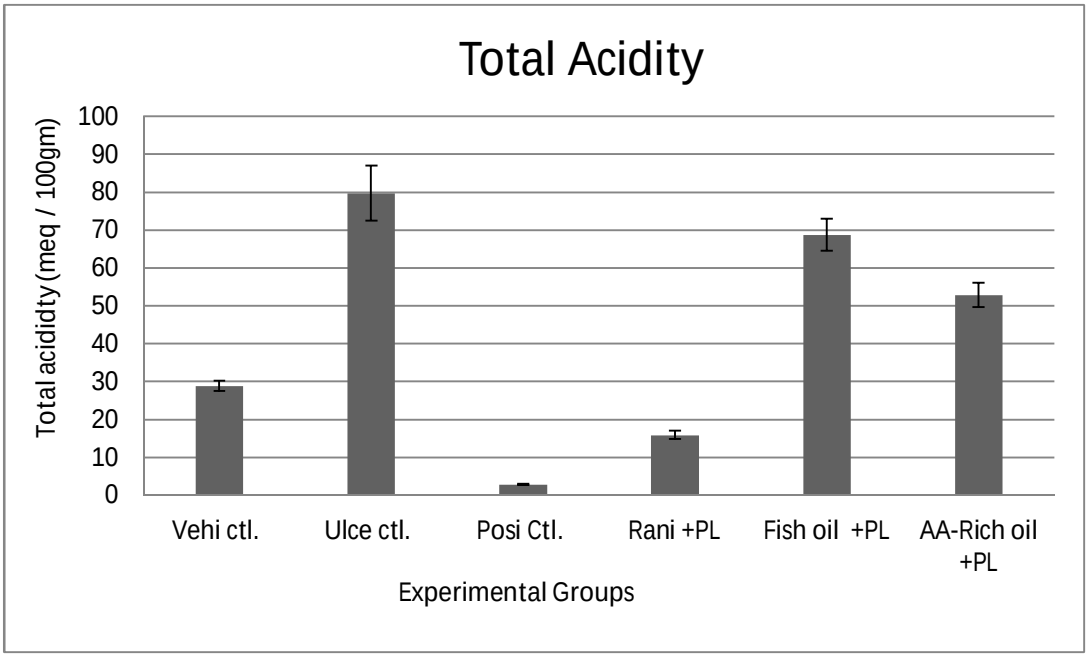
Figure 25: FO and AA on free acidity in pylorus ligated rats (n=6)

Effect of fish oil and Arasco oil on total acidity

The total acidity of gastric juice of animals from untreated control groups was 28.89±1.34, whereas the total acidity in pylorus ligation induced ulcer induced animal group showed a value of 79.8±7.26 total acidity. Fish oil significantly reduced the total acidity compared to ulcerated controls with a value of 68.78 ± 4.24. Arasco oil was much efficient compared to fish oil with a free acidity value of 52.87 ± 3.15 The standard drug Ranitidine reduced the total acidity much lower than the untreated control with a value of 15.9 ± 1.07. Similarly, Omeprazole reduced the total acidity to a much less value of 2.78 ± 0.15 meq/100gm.

Table 15: Effect of fish oil and Arasco oil on total acidity in pylorus ligated rats (n=6)

Group No.	Treatment (mg/kg)	Total acidity (Mean ± SEM)
Gp- I	Vehicle	28.89±1.34
Gp-II	Ulcerated control (pyloric ligation)	79.8±7.26
Gp- III	Omeprazole (20mg/kg, p.o.)	2.78 ± 0.15
Gp-IV	Ranitidine (30mg/kg, p.o.), + pyloric ligation.	15.9 ± 1.07
Gp- V	Fish oil (40µl/day, p.o.) + pyloric ligation.	68.78 ± 4.24
Gp-VI	AA-Rich oil (40µl/day, p.o.) + pyloric ligation.	52.87 ± 3.15



*Values are expressed as mean ± SEM (n=6), * p<0.05 and ** p<0.01 In comparison to control group with ulcers.*

Figure 26: FO and AA on total acidity in pylorus ligated rats (n=6)

6.4 INDOMETHACIN PLUS HISTAMINE

Introduction

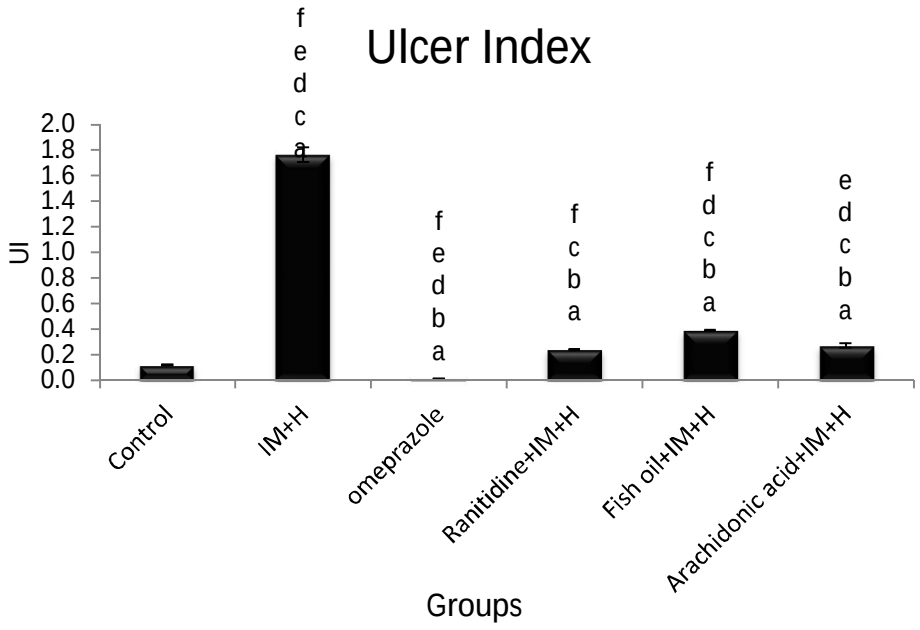
The practice of using a combination of indomethacin with histamine to induce gastric ulcers is a popular method. Brown et al., were the pioneers in introducing the method and partly explained the mechanism (89). They explained the involement of H1 and H2 histamine receptor agonists which aggrevated the ulceration produced by indomethacin. The current method of indomethacin plus histamine was first introduced by Takeuchi et al (68). Since then, the methods has gained popularity. We tested our hypothesis that fish oil and arachidonic acid rich oil would prevent gastric ulcer formation in indomethacin plus histamine induced gastric ulcer model.

Ulcer index:

Ulcer index is a measure of the extent of ulcer induced tissue damage. The combination of indomethacin and histamine induced a damge with an ulcer index of 1.8±.057 whereas the control showed an index of merely 0.1±.007. On the other hand, fish oil could control the damage induced by indomethacin plus histamin effectively with an ulcer index of 0.4±.005 Similarly, arachidonic acid could effectively reduce the ulcer with an index of 0.3±.017. The standard drug Ranitidine could offer a protection with an ulcer index of 0.2±.006. Omeprazole was still effective in control background with an ulcer index of 0.0±.001. Overall, our experiments prove that fish oil and arachidonic acid could effectively protect indomethacin plus histamine induced ulcer.

Table16: FO and AA on UI in indomethacin+histamine (n=6)

group No.	Treatment (mg/kg)	Ulcer index Mean ± SEM
Gp –I	Control	0.1±.007
Gp-II	Ulcerated control (indomethacin 5mg/kg+histamine 40mg/kg)	$1.8\pm.057^{acdef}$
Gp-III	Omeprazole 20mg/kg	$0.0\pm.001^{abdef}$
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	$0.2\pm.006^{abcf}$
Gp-V	Fish oil (40µl/ml/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	$0.4\pm.005^{abcdf}$
Gp-VI	Arachidonic acid (40µl/ml/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	$0.3\pm.017^{abcdef}$



Mean ± SEM (n=6). The SS of the data is p<0.05. a-C vs. O; b-IM+H vs. others; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

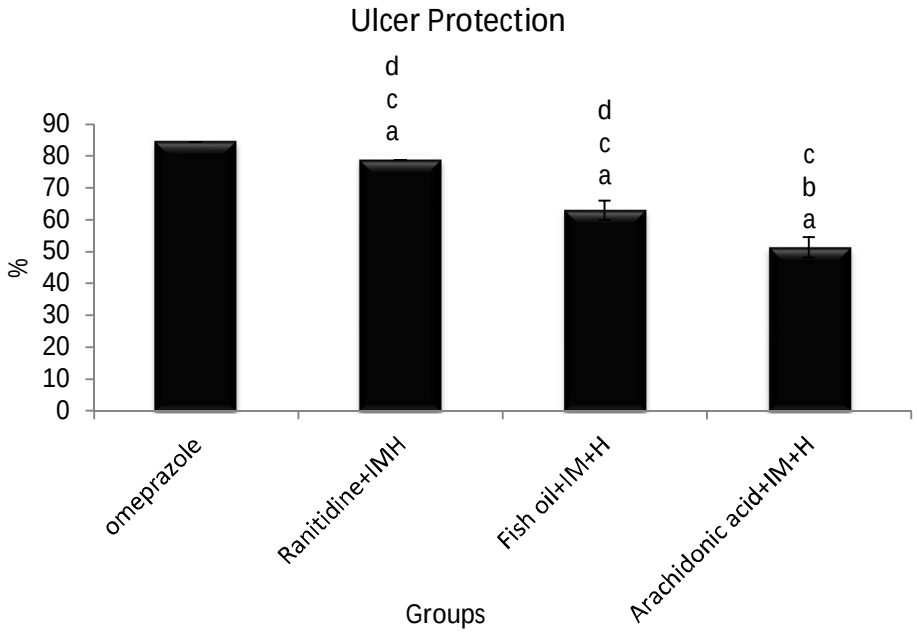
Figure 27: FO and AA on UI in indomethacin+histamine

Gastro protection of ulcer:

The protective effect of the test compounds were analysed and given as percentage protection. The results revealed that fish oil could protect with an ulcer index of 63±2.362. Arachidonic acid could protect ulcer with an ulcer index of 51.333±2.040. The standard drug Ranitidine, the standard drug protected the ulcer with a maximum index of 78.833±3.146. Omeprazole without induction of ulcer protected the ulcer with an index of 84.5±3.085. Collectively, the results suggest that both fish oil as well arachidonic acid protected animals from gastric ulcer formation with varying degrees of ulcer indices.

Table 17: FO and AA on % of gastroprotection in indomethacin+histamine (n=6)

group No.	**Treatment (mg/kg)**	**% of gastro protection Mean ± SEM**
Gp –I	Control	0
Gp-II	Ulcerated control(indomethacin 5mg/kg+histamine 40mg/kg)	0
Gp-III	Omeprazole 20mg/kg	84.5±3.085
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	78.833±3.146^{acd}
Gp-V	Fish oil (40µl/ml/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	63±2.362^{acd}
Gp-VI	Arachidonic acid (40µl/ml/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	51.333±2.040^{abc}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-C vs. O; b-IM+H vs. O; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

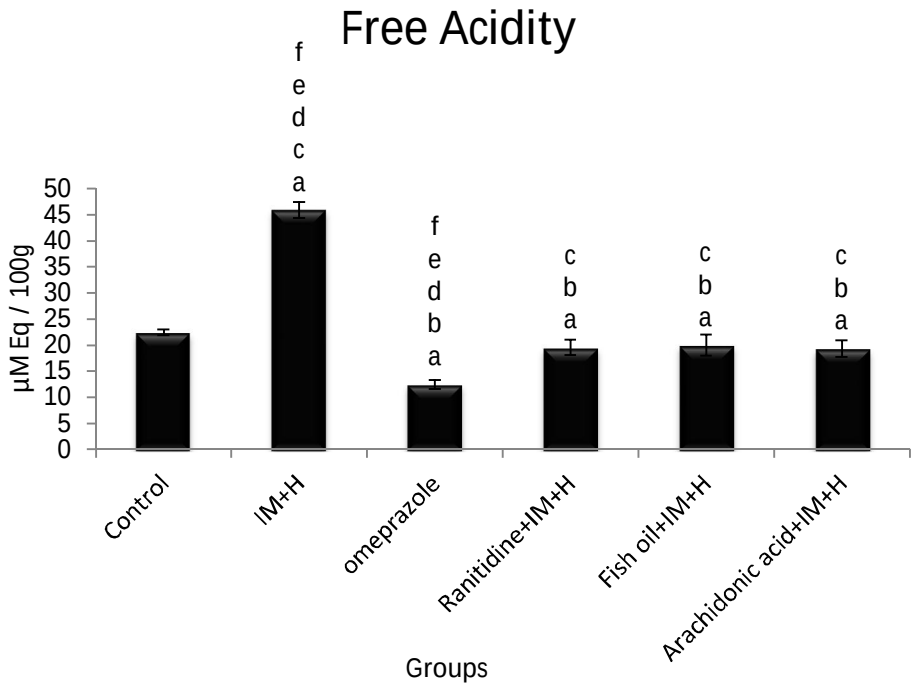
Figure 28: FO and AA on % of gastroprotection in indomethacin+histamine (n=6)

Stomach free acidity:

Free acidity, is involved in gastric ulcer formation. Therefore we measured the free acidity in all the groups of indomethacin plus histamine induced ulcer model. Free acidity in control measured with a value of 22.43 ±0.566. In indomethacin plus histamine induced ulcer group the free acidity was around 45.917±1.508. Fish oil protected the free acidity formation with a value of 20±1.966. Similarly, arachidonic acid also protected free acidity formation with a value of 19.333±1.606. The standard drug Ranitidine, effectively reduced the free acidity with a value of 19.533±1.480. Omeprazole, with no ulcer background reduced the free acidity to as low as 12.5±0.847 which is significantly lesser than control groups. On a whole, the protection from ulcer formation by fish oil or arachidonic acid was attributable to, at least in part, by controlling the free acidity.

Table 18: ***FO and AA on free acidity in indomethacin+histamine induced gastric ulcer in rats (n=6)***

Group No.	Treatment (mg/kg)	Free acidity Mean ± SEM
Gp -I	Control	22.43 ±0.566
Gp-II	Ulcerated control(indomethacin 5mg/kg+histamine 40mg/kg)	45.917±1.508 ^{acdef}
Gp-III	Omeprazole 20mg/kg	12.5±0.847^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	19.533±1.480^{abc}
Gp-V	Fish oil(40µl/ml/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	20.1 ±1.966^{abc}
Gp-VI	Arachidonic acid (40µl/ml/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	19.333 ±1.606^{abc}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Cvs. O; b-IM+H vs. O; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

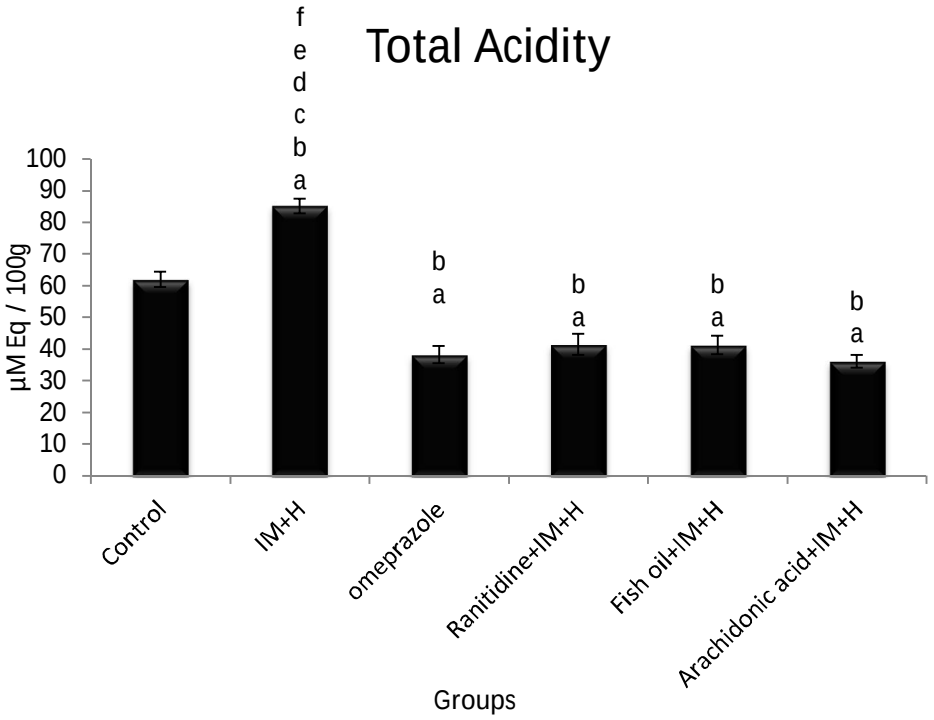
Figure 29: ***FO and AA on free acidity in indomethacin+histamine induced gastric ulcer in rats(n=6)***

Stomach Total acidity:

Total acidity in control was measured to be around 62 ± 2.394 and in ulcerated control the total acidity was measured to be around 85.167±2.386. Fish oil and Arachidonic acid was effective in reducing the total acidity contributed by indomethacin and arachidonic acid with values 41.333±2.883 and 36.167±1.973. The standard drug Ranitidine reduced the total acidity to 41.5±3.403. Omeprazole in an untreated control background protected free acidity formation with a value of 38.333±2.716. Therefore, the results indicate that fish oil and arachidonic acid were much efficient than the standard drug Ranitidine in controlling the total acidity.

Table 19: FO and A on total acidity in indomethacin+histamine (n=6)

group No.	**Treatment (mg/kg)**	**Total acidity Mean ± SEM**
Gp –I	Control	62 ± 2.394
Gp-II	Ulcerated control(indomethacin 5mg/kg+histamine 40mg/kg)	85.167 ± 2.386^{abcdef}
Gp-III	Omeprazole 20mg/kg	38.333 ± 2.716^{ab}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	41.5 ± 3.403^{ab}
Gp-V	Fish oil (40µl/ml/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	41.333 ± 2.883^{ab}
Gp-VI	Arachidonic acid (40µl/ml/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	36.167 ± 1.973^{ab}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Cvs. O; b-IM+H vs. O; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

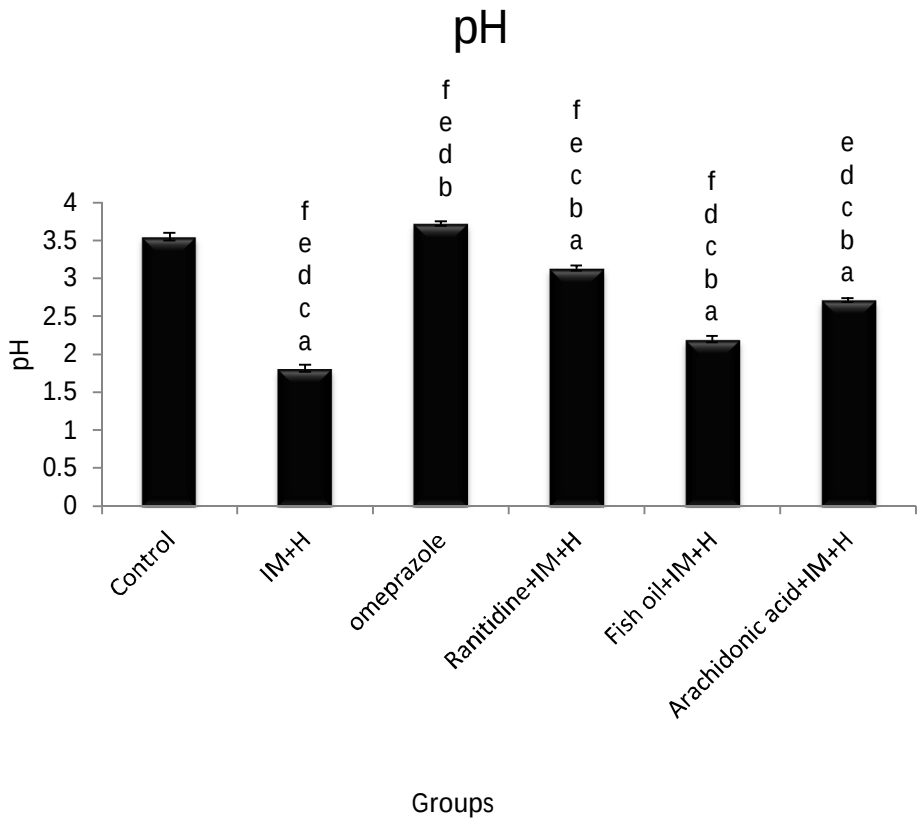
Figure 30: FO and AA on total acidity in indomethacin+histamine (n=6)

Gastric pH:

Measurement of the pH of gastric juice is a direct measure of the acidity of the gastric content. Therefore, we performed assessing the pH of gastric juice. The control showed a pH of around 3.55±0.049 whereas, untreated ulcerated control induced by indomethacin plus histamine reduced the pH to as low as 1.815 ±0.046. Fish oil and arachidonic acid could restore the pH of gastric juice with values 2.2 ±0.039 and 2.718 ±0.026. Among these two, arachidonic acid was comparatively better in restoring the pH. The standard drug Ranitidine, restored the pH near to control with a pH of 3.133 ±0.034. Omeprazole, in control background reduced the pH further to 3.723 ±0.030.

Table 20: FO and AA on pH in indomethacin+histamine (n=6)

group No.	Treatment (mg/kg)	pH Mean ± SEM
Gp –I	Control	3.55±0.049
Gp-II	Ulcerated control(indomethacin 5mg/kg+histamine 40mg/kg)	1.815 ± 0.046^{acdef}
Gp-III	Omeprazole 20mg/kg	3.723 ± 0.030^{bdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	3.133 ± 0.034^{abcef}
Gp-V	Fish oil (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	2.2 ± 0.039^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine(40mg/kg)	2.718 ± 0.026^{abcde}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Cvs. O; b-IM+H vs. O; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

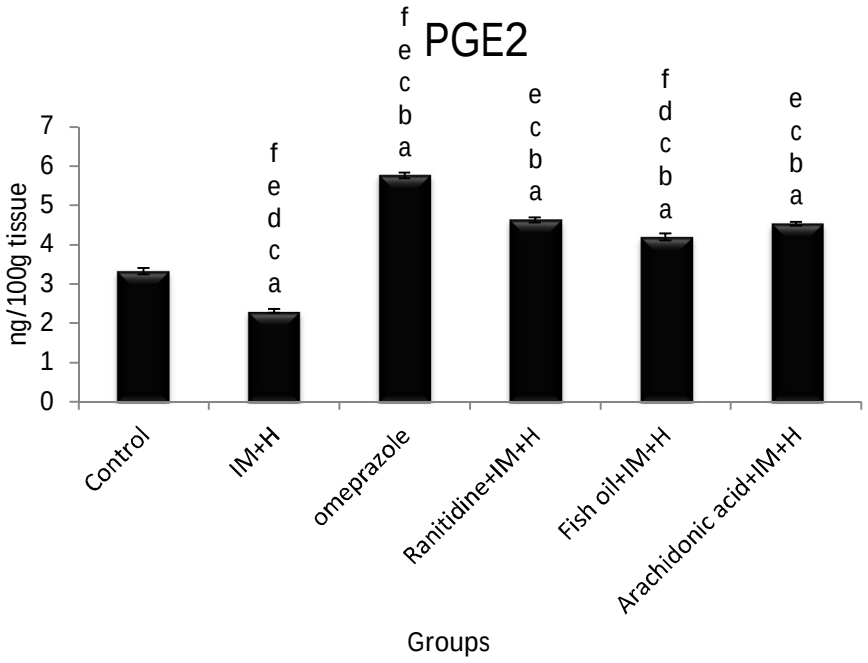
Figure 31: FO and AA on pH in indomethacin+histamine (n=6)

Expression of PGE_2

Prostaglandin has been well known as a suppressive agent for acid-secretion and thereby preventing gastric mucosa (90). We therefore estimated the level of prostaglandin E_2 in all the animal groups. In control group, the PGE_2 level was estimated to be around 3.325±0.077 and upon challenging with indomethacin along with histamine PGE_2 levels dropped to 2.31±0.0478. Upon treating the animals with fish oil and Arasco oil the PGE_2 levels increased drastically to 4.19±0.089 and 4.5317±0.043 respectively. Ranitidine, the standard drug, induced the expression of PGE_2 to around 4.628±0.066. On the other hand, Omeprazole treatment elevated the expression of PGE_2 to around 5.755±0.075. Overall, the present assay revealed that fish oil and Arasco oil both were effective in elevating prostaglandin E_2 level to minimize the indomethacin plus histamine-induced gastric ulcer.

Table 21: FO and AA on PGE_2 in indomethacin+histamine induced gastric ulcer in rats(n=6)

group No.	**Treatment (mg/kg)**	**PGE_2 Mean ± SEM**
Gp –I	Control	3.325±0.077
Gp-II	Ulcerated control (indomethacin 5mg/kg+histamine 40mg/kg)	$2.31±0.0478^{acdef}$
Gp-III	Omeprazole 20mg/kg	$5.755±0.075^{abcef}$
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	$4.628±0.066^{abce}$
Gp-V	Fish oil (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	$4.19±0.089^{abcdf}$
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	$4.5317±0.043^{abce}$



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Cvs. O; b-IM+H vs. O; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

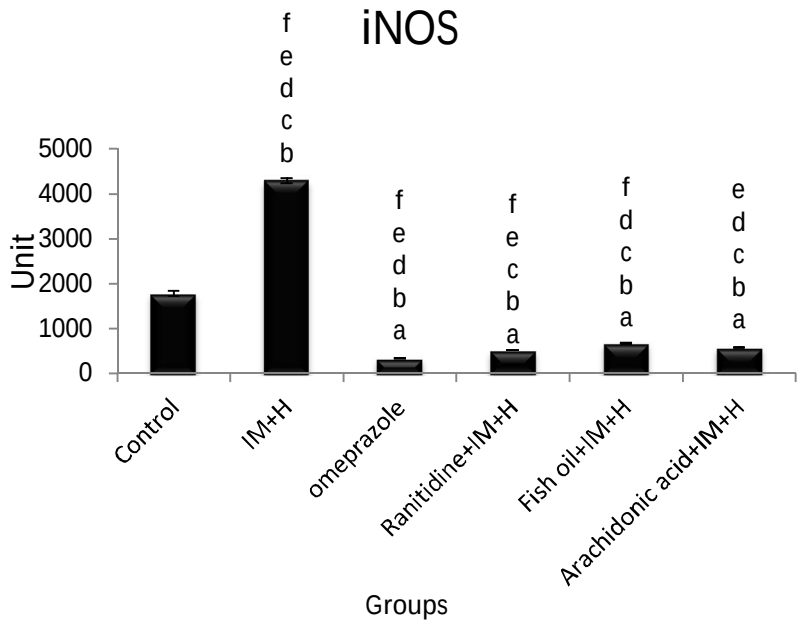
Figure 32: FO and AA on PGE_2 in indomethacin+histamine induced gastric ulcer in rats (n=6)

Expression of iNOS

Nitric oxide (NO) has a protective function in the gastric mucosa (91).In addition, Khattab et al., showed that nitric oxide also has mechanisms other than controlling gastric acid secretion (92). Therefore we examined the inducible nitric oxide synthase, an enzyme primarily involved in the synthesis of nitric oxide. In control group, iNOS level was observed to be 1784.333±52.509 and in indomethacin plus histamine treated group it elevated the expression of iNOS to around 4293.833±52.376. Surprisingly, in fish oil and arachidonic acid treated groups, iNOS level was reduced to 676.333±11.837 and 580.833±12.300. Ranitidine, the standard drug reduced the expression of iNOS was 519.833±2.8333. Omeprazole, in untreated control background had a reduced level iNOS with a value of 339.5 ±5.303.

Table 22: FO and AA on iNOS in indomethacin+histamine (n=6)

group No.	Treatment (mg/kg)	iNOS Mean ± SEM
Gp –I	Control	1784.333±52.509
Gp-II	Ulcerated control(indomethacin 5mg/kg+histamine 40mg/kg)	4293.833±52.376^{acdef}
Gp-III	Omeprazole 20mg/kg	339.5 ±5.303^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	519.833± 2.8333^{abcef}
Gp-V	Fish oil(40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	676.333±11.837^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	580.833 ±12.300^{abcde}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Cvs. O; b-IM+H vs. O; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

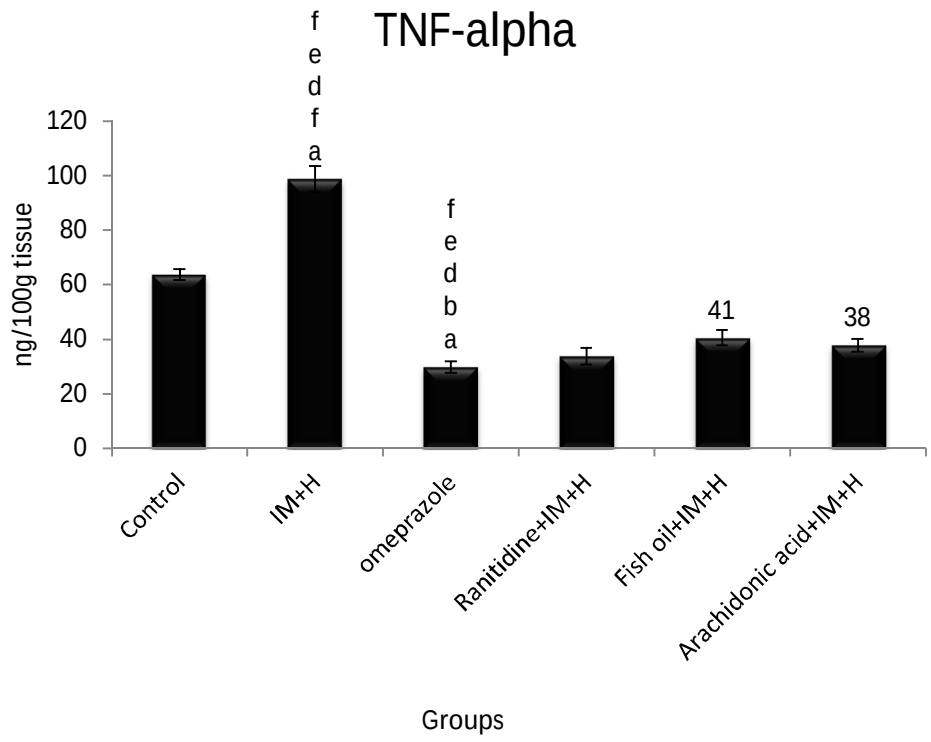
Figure 33: FO and AA on iNOS in indomethacin+histamine (n=6)

Expression of TNFα

It has long been known that elevation of TNF-α has a protective effect in gastric ulcer formation (93). We therefore, checked the levels of TNF-α for its role in ulcer protective ability in indomethacin plus histamine induced animal models. Control groups showed a level of 64±1.994 and upon challenging with indomethacin plus histamine the level rose to 99±4.695. Fish oil treatment decreased the TNF-α level to 41±2.814. Arachidonic acid also reduced the level of TNF-α to around 38±2.414. The standard drug, Ranitidine, was much potent in reducing the TNF-α level to 34±3.070. Omeprazole in untreated control background reduced the expression of TNF-α to 30±2.197.

Table 23: FO and AA on TNF-alpha in indomethacin+histamine (n=6)

group No.	**Treatment (mg/kg)**	**TNF-alpha Mean ± SEM**
Gp –I	Control	64±1.994
Gp-II	Ulcerated control(indomethacin 5mg/kg+histamine 40mg/kg)	99 ± 4.695^{acdef}
Gp-III	Omeprazole 20mg/kg	30 ± 2.197^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	34±3.070
Gp-V	Fish oil(40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	41±2.814
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	38±2.414



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Cvs. O; b-IM+H vs. O; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

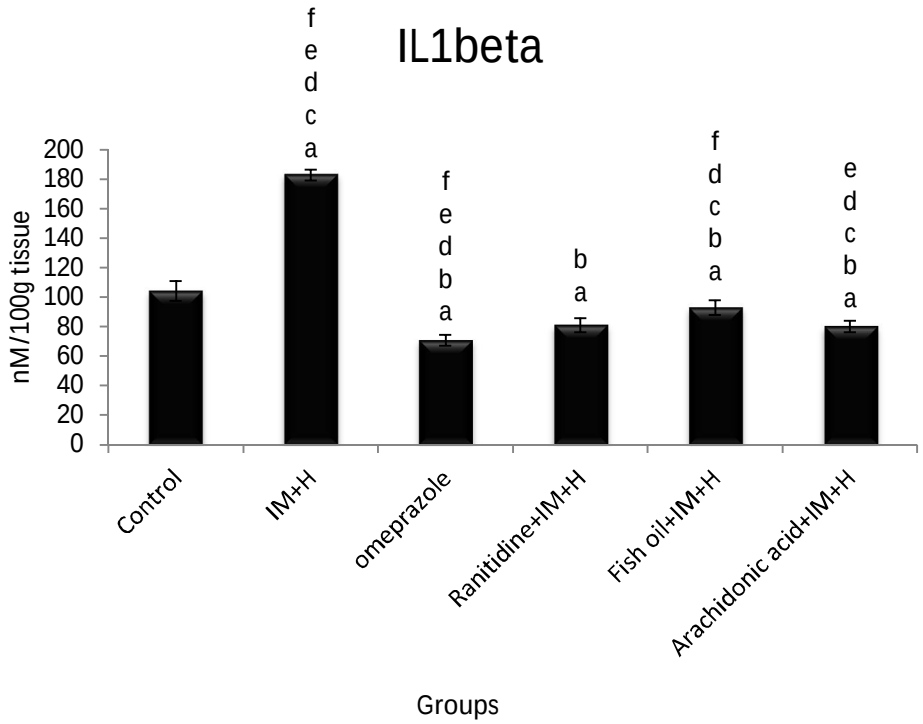
Figure 34: FO and AA on TNF-alpha in indomethacin+histamine (n=6)

Expression of IL-1

Interleukin-1 has been well known to be an anti-secretory and anti-ulcerative by acting on brain(94- 95). In control group the IL-1 level was 104±6.730 and indomethacin plus histamine treatment elevated the level of IL-1 to 183±3.596. Treatment with fish oil and arachidonic acid reduced the IL-1 level to 93±5.170 and 80±3.945 respectively. Ranitidine, the standard drug lowered IL-1 level to 81±4.833 and Omeprazole in control background reduced the level of IL-1to 71±3.697. These results indicate that the lower expression of IL-1 in fish oil and arachidonic acid treated animals is an indication that the ulcer formation is prevented in these groups.

Table 24: FO and AA on IL1 in indomethacin+histamine (n=6)

group No.	Treatment (mg/kg)	IL1 Mean ± SEM
Gp –I	Control	104±6.730
Gp-II	Ulcerated control(indomethacin 5mg/kg+histamine 40mg/kg)	183±3.596^{acdef}
Gp-III	Omeprazole 20mg/kg	71±3.697^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	81±4.833^{ab}
Gp-V	Fish oil (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	93±5.170^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	80±3.945^{abcde}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Cvs. O; b-IM+H vs. O; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

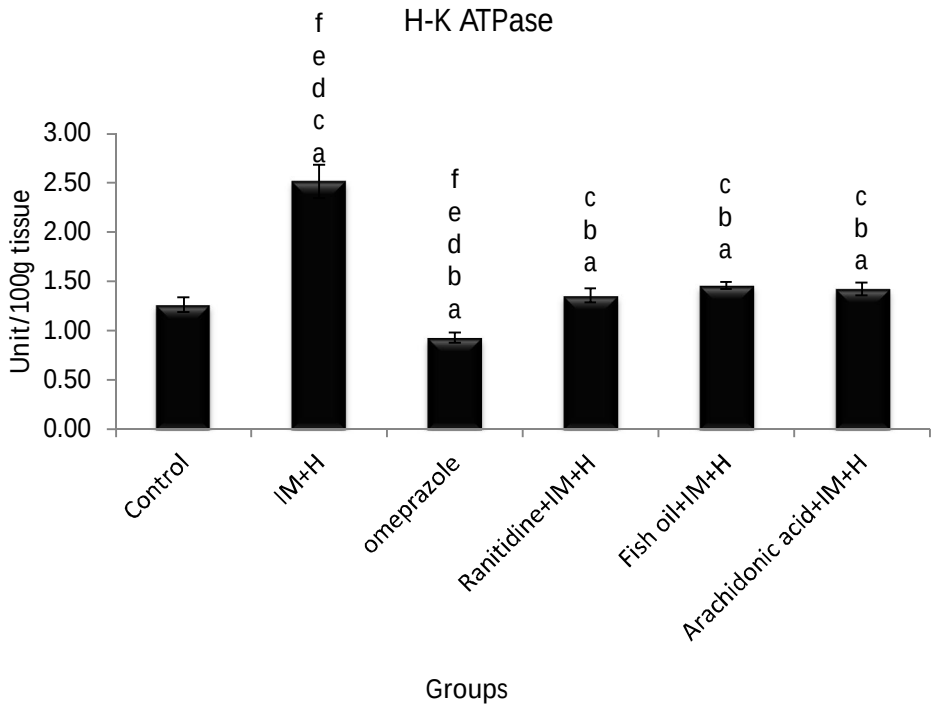
Figure 35: FO and AA on TNF-alpha in indomethacin+histamine (n=6)

H+K+ATPase:

Maintenance of acidic pH of the stomach is achieved by gastric H^+/K^+ ATPase proton pump which lies at the end of all pathways stimulating gastric acid production (96). Therefore, we examined the proton pump in our animal groups. In control group, the amount of H^+/K^+ ATPase was around 1.27±.074. Ulcer induced animals showed an elevated level of H^+/K^+ ATPase proton pump with a value of 2.52±.169. Fish oil treated animals showed a level of 1.46±.034H^+/K^+ ATPase proton pump. Similarly, arachidonic acid treated group of animals displayed a level of 1.42±.066. The standard drug, Ranitidine showed a much protective effect with a reduced value of 1.36±.071. Omeprazole in control background resulted in 0.93 ±.050 of H^+/K^+ ATPase proton pump.

Table 25: FO and AA on H+K+ATPase in indomethacin+histamine (n=6)

group No.	**Treatment (mg/kg)**	**H+K+ATPase Mean ± SEM**
Gp –I	Control	1.27±.074
Gp-II	Ulcerated control(indomethacin 5mg/kg+histamine 40mg/kg)	$2.52±.169^{acdef}$
Gp-III	Omeprazole 20mg/kg	$0.93 ±.050^{abdef}$
Gp-IV	Ranitidine (30mg/kg.p.o)prior to(indomethacin 5mg/kg+histamine 40mg/kg)	$1.36±.071^{abc}$
Gp-V	Fish oil (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	$1.46±.034^{abc}$
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by (indomethacin 5mg/kg+histamine 40mg/kg)	$1.42±.066^{abc}$



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Cvs. O; b-IM+H vs. O; c-Omeprazole vs others, d-Ranitidine+IM+H vs others; e-Fish oil+IM+H vs others; f-AA+IM+H vs others

Figure 36: FO and AA on H+K+ATPase in indomethacin+histamine (n=6)

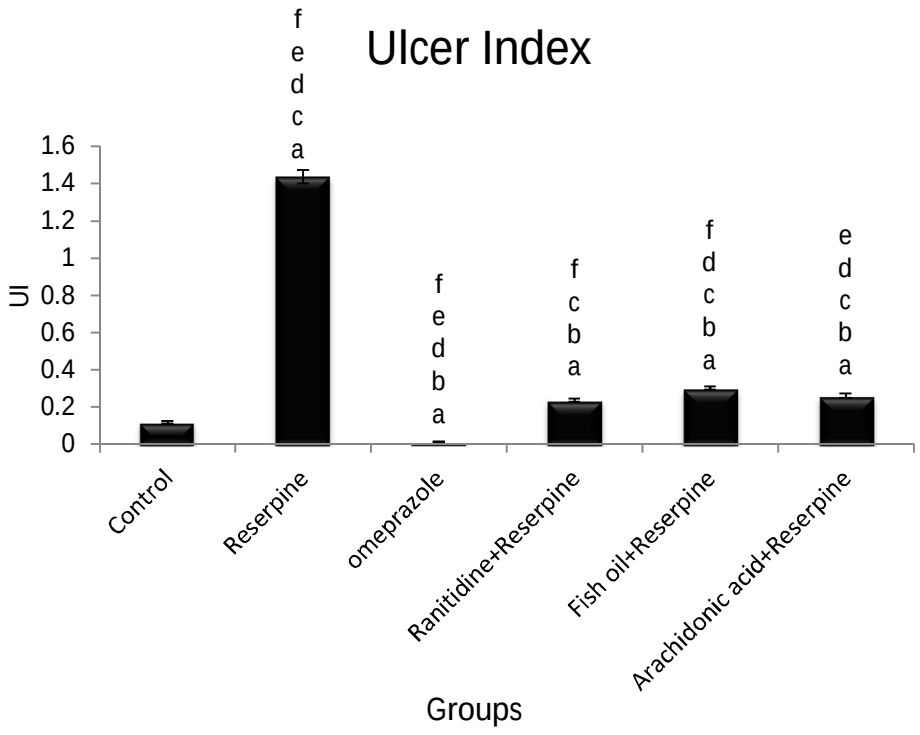
6.5 RESERPINE INDUCED ULCERS

Ulcer Index

The ulcer induced by Reserpine was around 1.438±0.036 whereas the control group showed an ulcer index of 0.120±0.007. Treatment with fish oil resulted in an ulcer index of 0.302±0.009. Upon treatment with arachidonic acid showed a value of 0.26±0.012. Ranitidine, the standard drug protected the ulcer induction with an ulcer index much closer to the control of 0.237±0.010. Omeprazole with a background of control reduced the ulcer index to about 0.013±0.001.

Table 26: FO and AA on ulcer index in reserpine (n=6)

Group No.	Treatment (mg/kg)	Ulcer index Mean ± SEM
Gp -I	Control	0.120±0.007
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	1.438±0.036^{acdef}
Gp-III	Omeprazole 20mg/kg	0.013±0.001^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Reserpine	0.237±0.010^{abcf}
Gp-V	Fish oil (40µl/day.p.o) followed by Reserpine	0.302±0.009^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by reserpine	0.26±0.012^{abcdef}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Cvs. O; b- Reserpine vs. O; c-Omeprazole vs others, d-Ranitidine+ Reserpine vs others; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others.

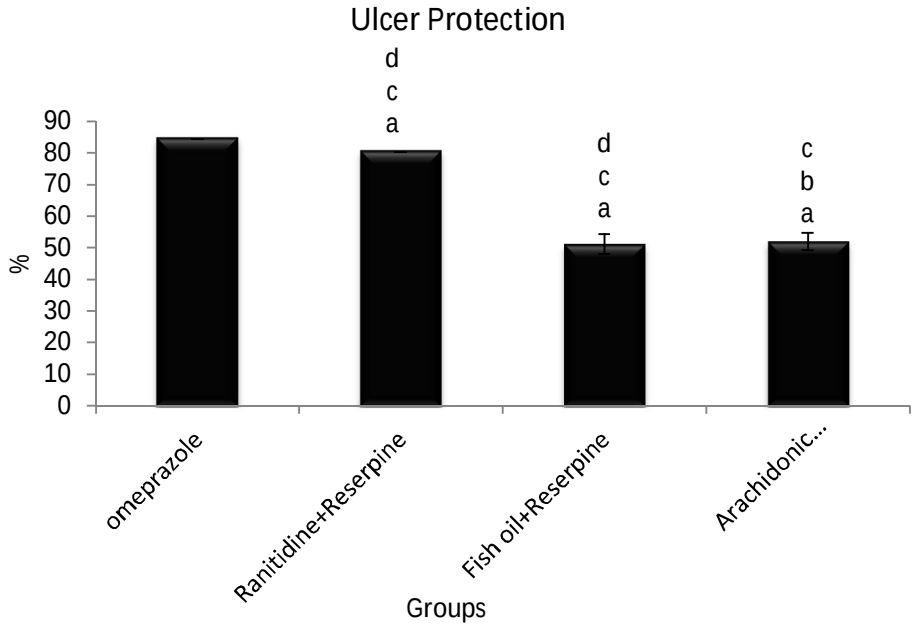
Figure 37: FO and AA on ulcer index in reserpine (n=6)

Gastro-protection:

Gastro protective ability determined by the ulcer index is given below. Control and ulcerated control showed no protection since no supplement was provided. Fish oil provided 51.167±2.810% protection whereas arachidonic acid provided 52±2.324% protection. Ranitidine, the standard compound provided 80.5±2.742% protection. Omeprazole which had no ulcer formation protected 84.5±3.085%

Table 27**: FO **and AA on% gastroprotection in reserpine (n=6).

Group No.	Treatment (mg/kg)	Ulcer protection Mean ± SEM
Gp –I	Control	0
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	0
Gp-III	Omeprazole 20mg/kg	84.5±3.085
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Reserpine	80.5 ± 2.742^{acd}
Gp-V	Fish oil (40µl/day.p.o) followed by Reserpine	51.167 ± 2.810^{acd}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Reserpine	52 ± 2.324^{abc}



Mean ± SEM (n=6). The SS of the data is p<0.05. a-Omeprazole vs O, d-Ranitidine+ Reserpine vs O; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others

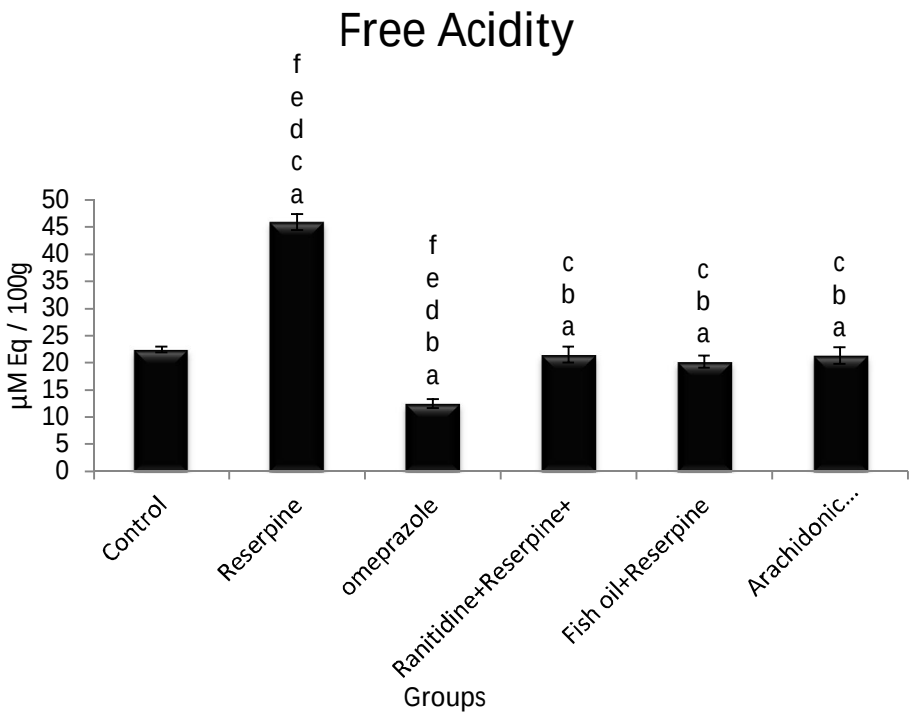
Figure 38: FO and AA on% gastroprotection in reserpine (n=6)

Free acidity:

Free acidity in control was measured to be around 22.433±0.566 and in reserpine induced ulcerated animals it was found to be 45.917±1.508. Fish oil protected the acidity formation and showed half the value of ulcerated animals with a value of 20.167±1.138. Similarly, arachidonic acid provided a reduction in pH with a value of 21.333±1.542. Ranitidine, the standard drug, reduced the pH to 21.5±1.478. Omeprazole, with a control background showed a free acidity of 12.5±0.847.

Table 28: FO and AA on free acidity in reserpine (n=6)

Group No.	**Treatment (mg/kg)**	**Free acidity Mean ± SEM**
Gp –I	Control	22.433±0.566
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	$45.917±1.508^{acdef}$
Gp-III	Omeprazole 20mg/kg	$12.5±0.847^{abdef}$
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Reserpine	$21.5±1.478^{abc}$
Gp-V	Fish oil (40µl/day.p.o) followed by Reserpine	$20.167±1.138^{abc}$
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Reserpine	$21.333±1.542^{abc}$



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b- Reserpine vs. O; c-Omeprazole vs others, d-Ranitidine+ Reserpine vs others; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others.

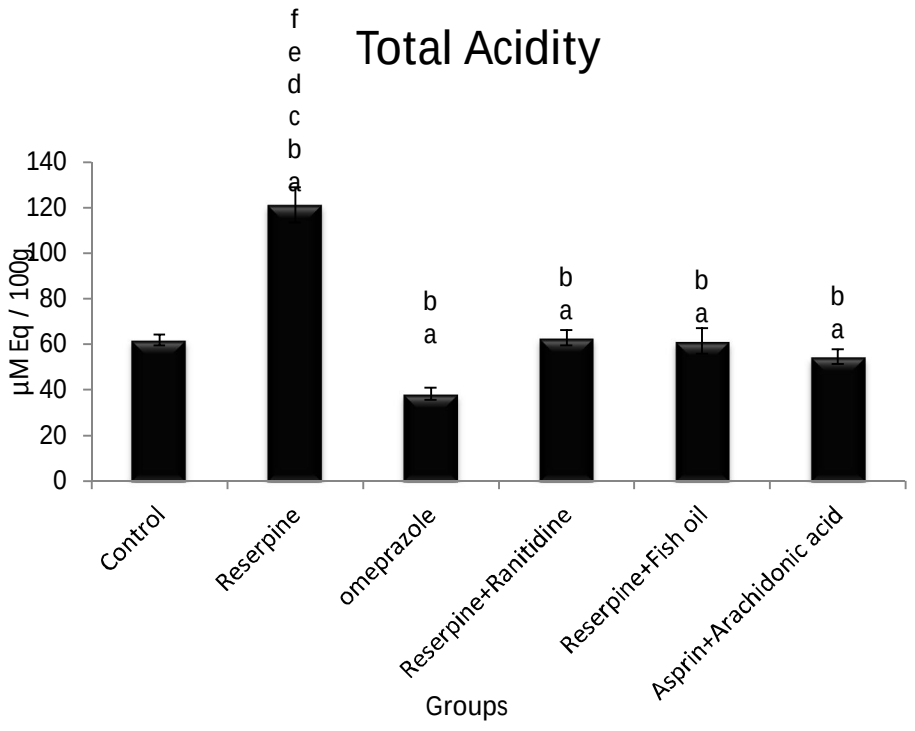
Figure 39: FO and AA on free acidity in reserpine (n=6)

Stomach Total acidity:

Total acidity in control was observed to be 62±2.394. Reserpine induced ulcerated animals showed a total acidity of 121.167±7.752. Total acidity dropped in the animals in which fish oil was treated and showed a total acidity of 61.5±5.638. Similarly, arachidonic acid treated animals showed a total acidity of 54.5±3.233. Standard drug Ranitidine treatment reduced the total acidity to 62.833±3.361. Omeprazole in control background showed a total acidity of 38.333±2.716

Table 29: FO and AA on total acidity in reserpine (n=6).

Group No.	Treatment (mg/kg)	Total acidity Mean ± SEM
Gp –I	Control	62±2.394
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	$121.167±7.752^{abcdef}$
Gp-III	Omeprazole 20mg/kg	$38.333±2.716^{ab}$
Gp-IV	Ranitidine (30mg/kg.p.o)prior to reserpine	$62.833±3.361^{ab}$
Gp-V	Fish oil(40µl/day.p.o) followed by reserpine	$61.5±5.638^{ab}$
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by reserpine	$54.5±3.233^{ab}$



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b- Reserpine vs. O; c-Omeprazole vs others, d-Ranitidine+ Reserpine vs others; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others.

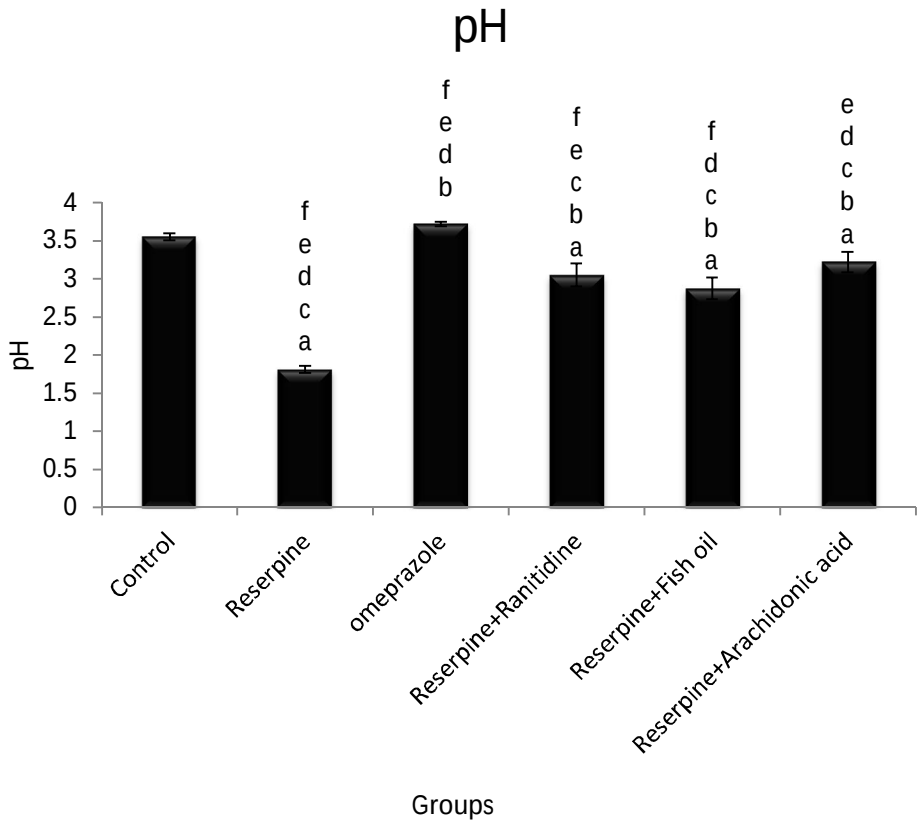
Figure 40: FO and AA on total acidity in reserpine (n=6).

Gastric pH:

Control animals displayed a pH of 3.55 ±0.04858 and reserpine induced animal groups had much acidic pH of 1.815 ±0.046. Fish oil prevented drop in pH with a value of 2.873 ±0.145 and arachidonic acid treatment further prevented further fluctuation in pH and stabilized with 3.22 ±0.138. Ranitidine on the other hand, showed only a pH of 3.05 ±0.152, which is comparatively lesser than the value given by arachidonic acid. Omeprazole, in control background provided a pH of 3.723 ±0.030

Table 30: FO and AA on pH in reserpine (n=6)

Group No.	**Treatment (mg/kg)**	**ph Mean ± SEM**
Gp –I	Control	3.55 ±0.04858
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	1.815 ±0.046^{acdef}
Gp-III	Omeprazole 20mg/kg	3.723 ±0.030^{bdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to reserpine	3.05 ±0.152^{abcef}
Gp-V	Fish oil (40µl/day.p.o) followed by reserpine	2.873 ±0.145^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by reserpine	3.22 ±0.138^{abcde}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b- Reserpine vs. O; c-Omeprazole vs others, d-Ranitidine+ Reserpine vs others; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others.

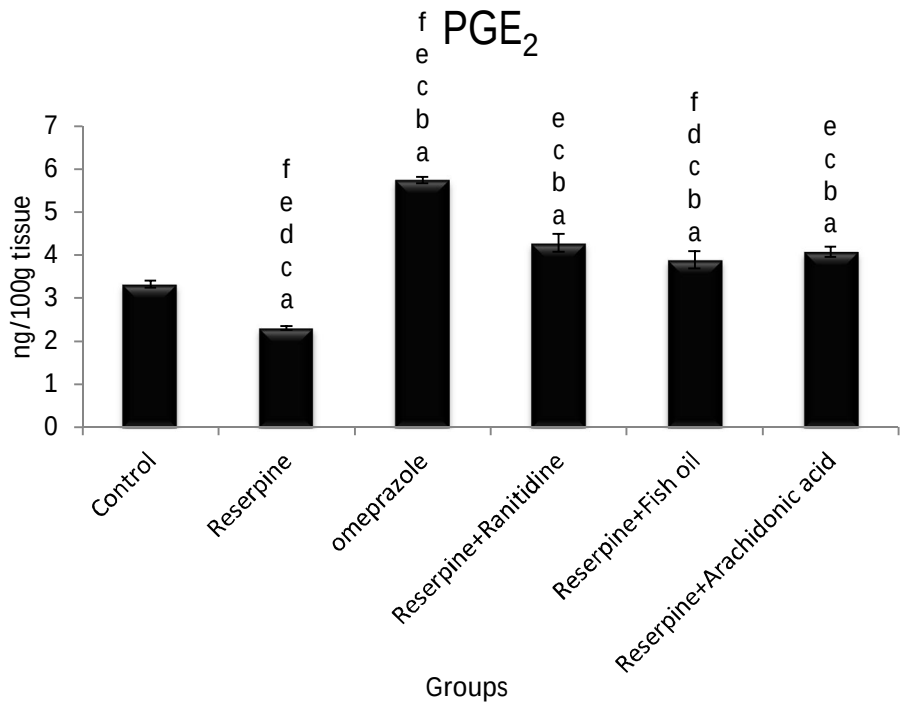
Figure 41: FO and AA on pH in reserpine (n=6)

Expression of Prostaglandin E_2:

Prostaglandin E_2 level was around 3.55 ±0.04858 in control group of animals. In ulcerated control with reserpine treatment, the value was 1.815 ±0.046. Treatment with fish oil slightly increased the prostaglandin E_2 level to around 2.873 ±0.145. Arachidonic acid treated animals showed further increased prostaglandin level with a value of 3.22 ±0.138. The standard drug Ranitidine, resulted in a level of 3.05 ±0.152, which is slightly lesser than the level achieved by arachidonic acid. Omeprazole, with control background provided a value of 3.723 ±0.030

Table 31: FO and AA on PGE_2 in reserpine (n=6)

Group No.	Treatment (mg/kg)	PGE_2 Mean ± SEM
Gp –I	Control	3.325±0.077
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	$2.31±0.0478^{acdef}$
Gp-III	Omeprazole 20mg/kg	$5.755±0.075^{abdef}$
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Reserpine	$4.283±0.209^{abce}$
Gp-V	Fish oil (40µl/day.p.o) followed by Reserpine	$3.9±0.202^{abcdf}$
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Reserpine	$4.085±0.122^{abce}$



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b- Reserpine vs. O; c-Omeprazole vs others, d-Ranitidine+ Reserpine vs others; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others.

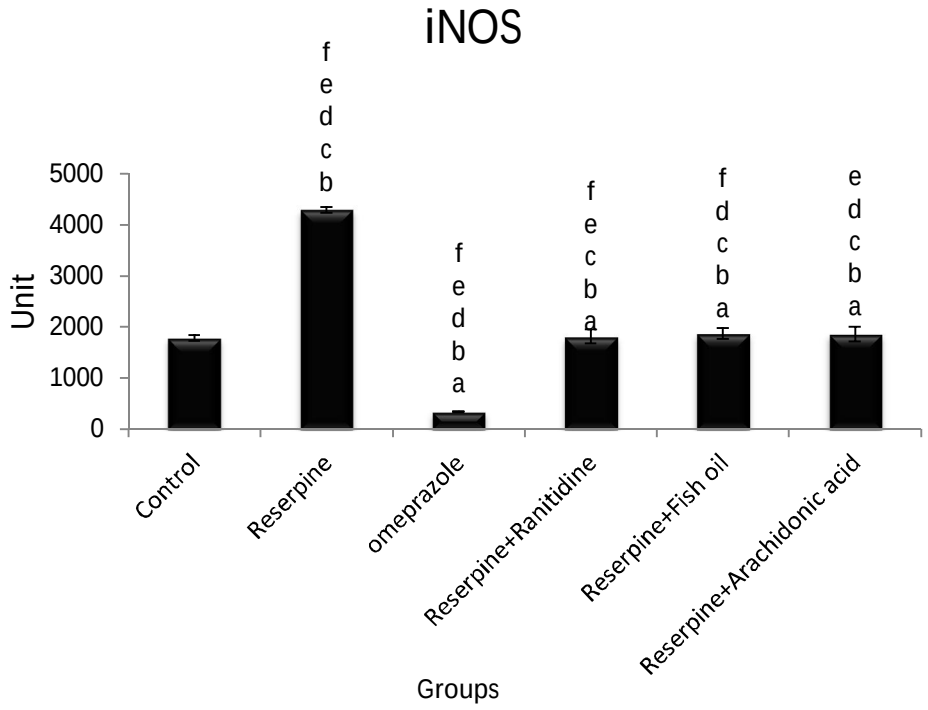
Figure 42: FO and AA on PGE_2 in reserpine (n=6)

Expression of iNOS

Inducible nitric oxide synthesis enzyme level in control animal was around 1784.333 ±52.509. Upon challenging with reserpine, ulcerated control group animals showed a level of 4293.833 ±52.376. Fish oil treatment reduced the iNOS level and had level of 1874.5 ±103.64. Similarly, arachidonic acid also reduced iNOS expression and showed 1857.167 ±141.751. Ranitidine, the standard drug also lowered the expression of iNOS with a value of 1814.333 ±142.237. Omeprazole with a control background, displayed a much reduced level of iNOS with a value of 339.5 ±5.303

Table 32: FO and AA on iNOS in reserpine (n=6)

Group No.	**Treatment (mg/kg)**	**iNOS Mean ± SEM**
Gp -I	Control	1784.333 ±52.509
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	4293.833 ±52.376^{bcdef}
Gp-III	Omeprazole 20mg/kg	339.5 ±5.303^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to reserpine	1814.333 ±142.237^{abcef}
Gp-V	Fish oil (40µl/day.p.o) followed by reserpine	1874.5 ±103.64^{abcef}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by reserpine	1857.167 ±141.751^{abcdef}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b- Reserpine vs. O; c-Omeprazole vs others, d-Ranitidine+ Reserpine vs others; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others.

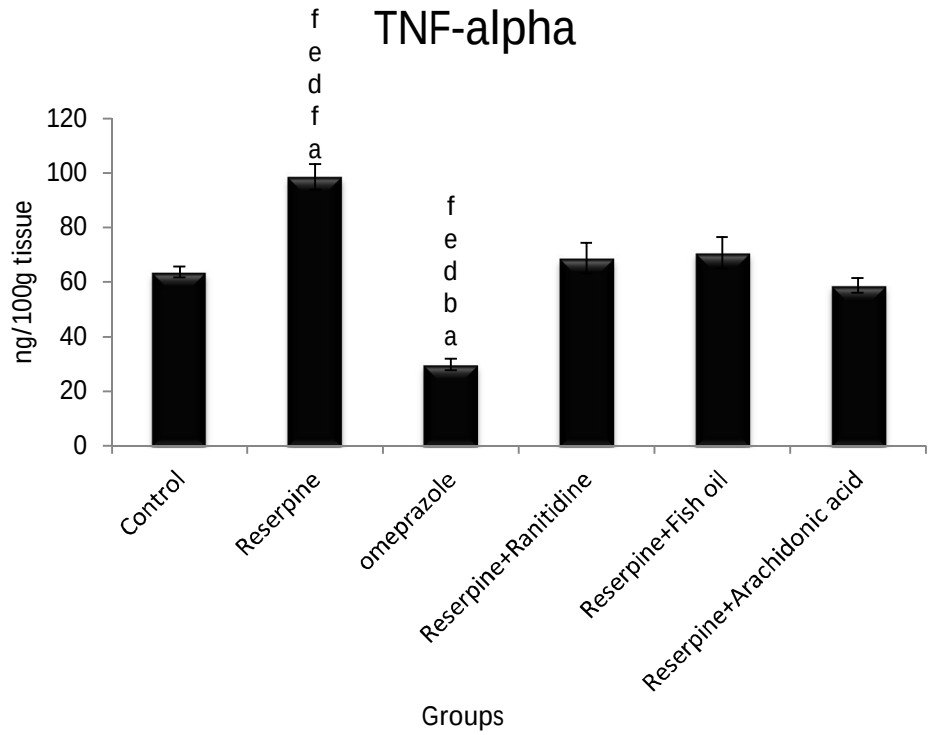
Figure 43: FO and AA on iNOS in reserpine (n=6)

Expression of TNF-α:

In control, TNF-α level was 63.667±1.994 and in ulcer induced animals the level was around 98.667±4.695. Fish oil reduced the expression of TNF-α to a value of 70.833±5.793. Similarly, arachidonic acid sharply reduced the expression of TNF-α to 58.833±2.774. Ranitidine, the standard drug restored the level of68.833±5.576. Omeprazole reduced the expression of TNF-α lesser than control with a value of 29.833±2.197.

Table 33: FO and AA on TNF-alpha in reserpine (n=6)

Group No.	Treatment (mg/kg)	TNF-alpha Mean ± SEM
Gp –I	Control	63.667±1.994
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	98.667±4.695afdef
Gp-III	Omeprazole 20mg/kg	29.833±2.197abdef
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Reserpine	68.833±5.576
Gp-V	Fish oil(40µl/day.p.o) followed by Reserpine	70.833±5.793
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Reserpine	58.833±2.774



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b- Reserpine vs. O; c-Omeprazole vs others, d-Ranitidine+ Reserpine vs others; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others.

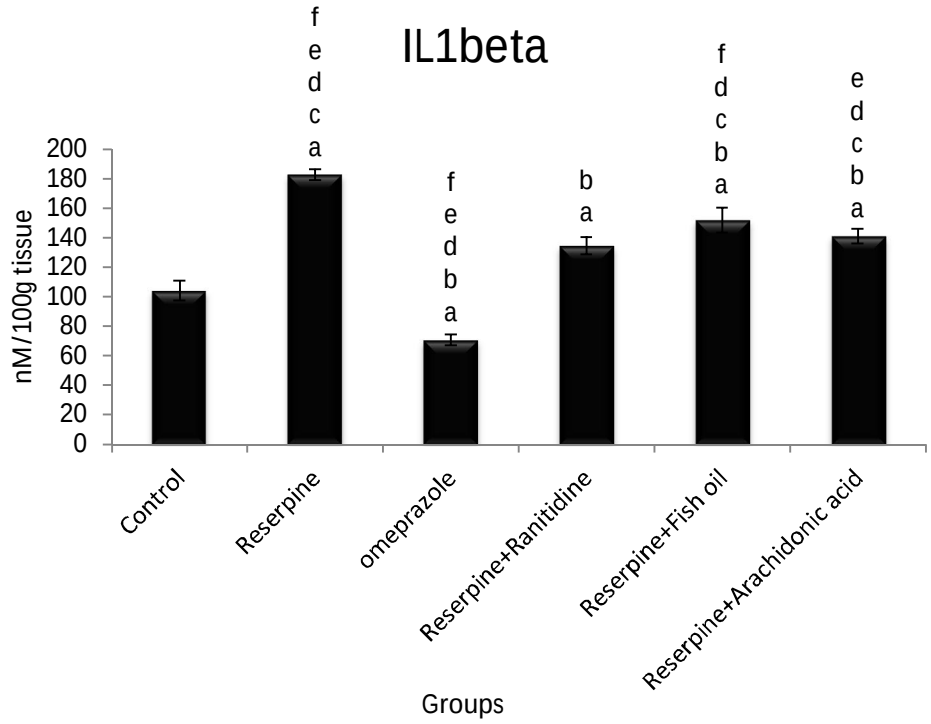
Figure44: FO and AA on TNF-alpha in reserpine (n=6)

Expression of IL-1β:

Interleukin-1β expression level was measured to be around 104.167 ±6.730 and reserpine treatment to induce ulcer elevated the level to 183 ±3.596. Fish oil treatment reduced the expression of Interleukin-1β to around 152 ±8.359. In a much similar manner, arachidonic acid also reduced the expression of Interleukin-1βto 141.167±5.029. The standard drug Ranitidine, reduced the interleukine-1βto much nearer to control with a value of 134.667 ±6.042. Omeprazole, reduced the expression of interleukin-1β to around 71 ±3.697.

Table 34: FO and AA on IL1beta in reserpine (n=6)

Group No.	**Treatment (mg/kg)**	**IL1 beta Mean ± SEM**
Gp -I	Control	104.167 ±6.730
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	183 ±3.596^{acdef}
Gp-III	Omeprazole 20mg/kg	71 ±3.697^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Reserpine	134.667 ±6.042^{ab}
Gp-V	Fish oil (40µl/day.p.o) followed by Reserpine	152 ±8.359^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Reserpine	141.167±5.029^{abcdef}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b- Reserpine vs. O; c-Omeprazole vs others, d-Ranitidine+ Reserpine vs others; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others.

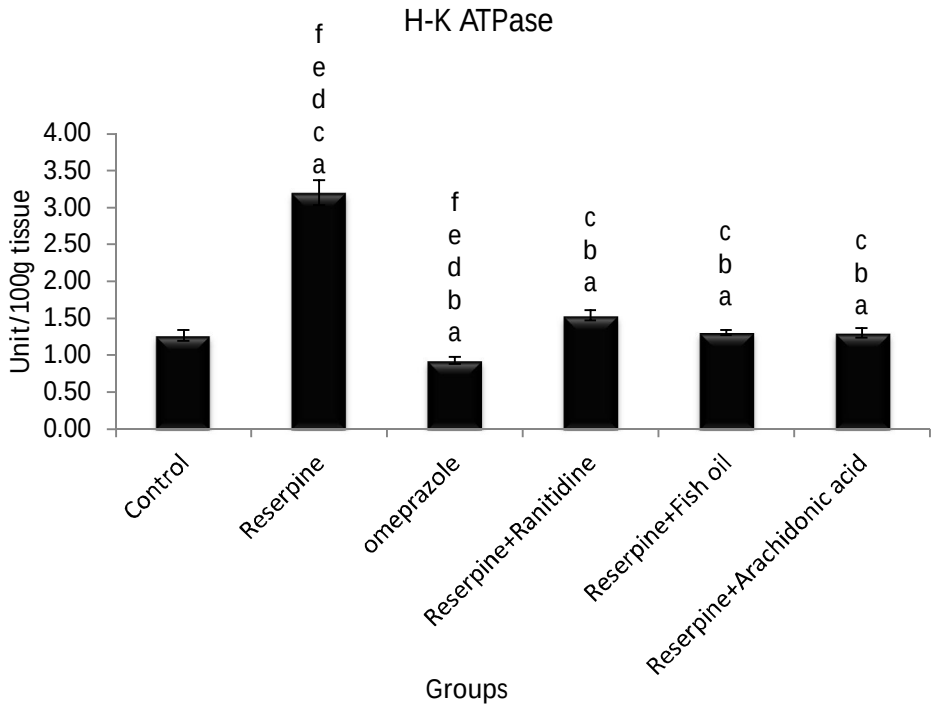
Figure 45: FO and AA on IL1beta in reserpine (n=6)

H^+/K^+ ATPase proton pump:

H^+/K^+ ATPase proton pump in control was 1.27±.074 and ulcer induction with reserpine induced the level to 3.20±.169. Fish oil treatment prevented the increase in H^+/K^+ ATPase proton pump with a value of 1.31±.034 and similarly, arachidonic acid also prevented the increase ofH^+/K^+ ATPase proton pump with a value of 1.30±.066. Ranitidine, increased the level of H^+/K^+ ATPase proton pump to 1.54±.071. Omeprazole, decreased the level of proton pump to 0.93±.050

***Table 35: FO and AA on H+K+ATPase in* reserpine (n=6)**

Group No.	Treatment (mg/kg)	H+K+ATPase Mean ± SEM
Gp -I	Control	1.27±.074
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	3.20±.169^{acdef}
Gp-III	Omeprazole 20mg/kg	0.93±.050^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Reserpine	1.54±.071^{abc}
Gp-V	Fish oil (40µl/day.p.o) followed by Reserpine	1.31±.034^{abc}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Reserpine	1.30±.066^{abc}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b- Reserpine vs. O; c-Omeprazole vs others, d-Ranitidine+ Reserpine vs others; e-Fish oil+ Reserpine vs others; f- AA+ Reserpine vs others.

Figure 46: FO and AA on H+K+ATPase in reserpine (n=6)

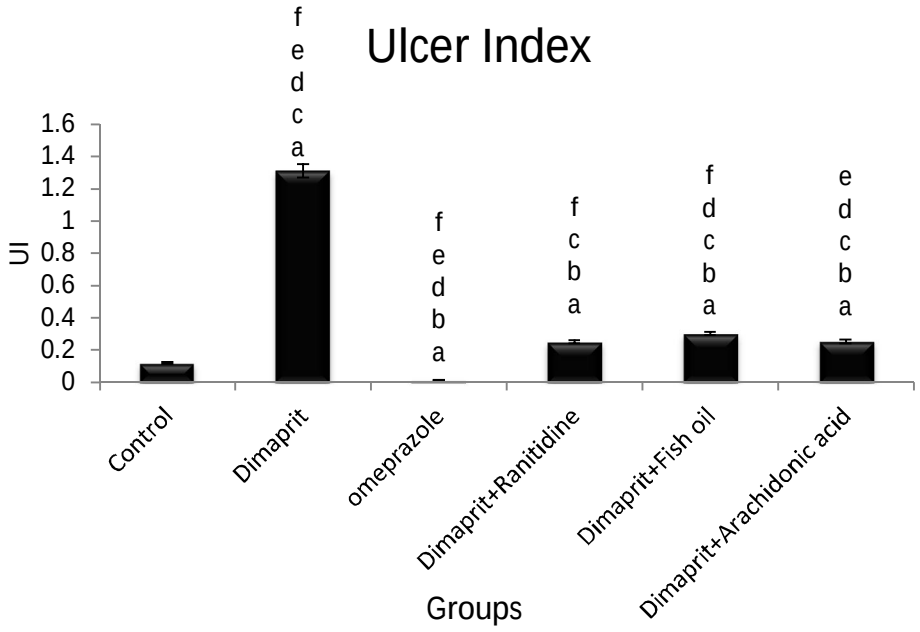
6.6 DIMAPRIT INDUCED DUODENAL ULCER

The control animals showed an ulcer index of 0.120±0.007. Dimaprit is a compound commonly used for induction of gastric ulcer. Ulcer index in Dimaprit induced ulcerated animals was found to be 1.312±0.042. The animals which were treated with fish oil prior to ulcer induction with Dimaprit showed an ulcer index of 0.302±0.009. Arachidonic acid also effectively reduced the ulcer index with a value of 0.254±0.012. The standard drug, Ranitidine reduced the ulcer index to 0.25±0.010. Omeprazole, in control background, showed an ulcer index of 0.013±0.001.

Dimaprit induced ulcer

Table 36: FO and AA on ulcer index in Dimaprit (n=6)

Group No.	Treatment (mg/kg)	Ulcer index Mean ± SEM
Gp –I	Control	0.120±0.007
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	1.312 ± 0.042^{acdef}
Gp-III	Omeprazole 20mg/kg	0.013 ± 0.001^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	0.25 ± 0.010^{abcf}
Gp-V	Fish oil (40µl/day.p.o) followed by Dimaprit	0.302 ± 0.009^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	0.254 ± 0.012^{abcde}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b-Dimaprit vs. O; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others bar.

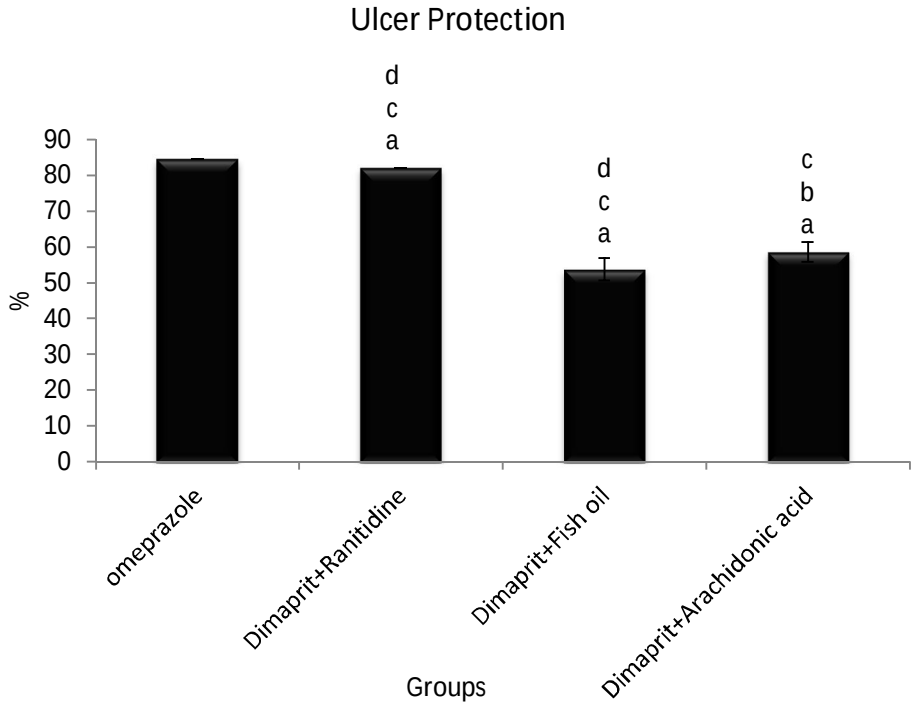
Figure 47: FO and AA on ulcer index in Dimaprit (n=6)

Gastro-protection:

The gastro protection was estimated from the ulcer index and expressed as percentage protection. The animals from ulcer induced group with Dimaprit showed no protection. Fish oil and arachidonic acid offered protection of 53.8±2.80971% and 58.6±2.32379% respectively. Ranitidine, the standard drug offered a maximum protection of 82.1±2.74165% whereas, Omeprazole in a control background showed a protection of 84.5±3.08491%.

Table 37: FO and AA on % gastro protection of in Dimaprit (n=6)

Group No.	Treatment (mg/kg)	%protection Mean ± SEM
Gp –I	Control	0
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	0
Gp-III	Omeprazole 20mg/kg	84.5±3.08491
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	82.1±2.74165^{acd}
Gp-V	Fish oil (40µl/day.p.o) followed by Dimaprit	53.8±2.80971^{acd}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	58.6±2.32379^{abc}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Omeprazole vs O, b-Ranitidine+Dimaprit vs O; c-Fish oil+ Dimaprit vs others; d- AA+ Dimaprit vs other bar

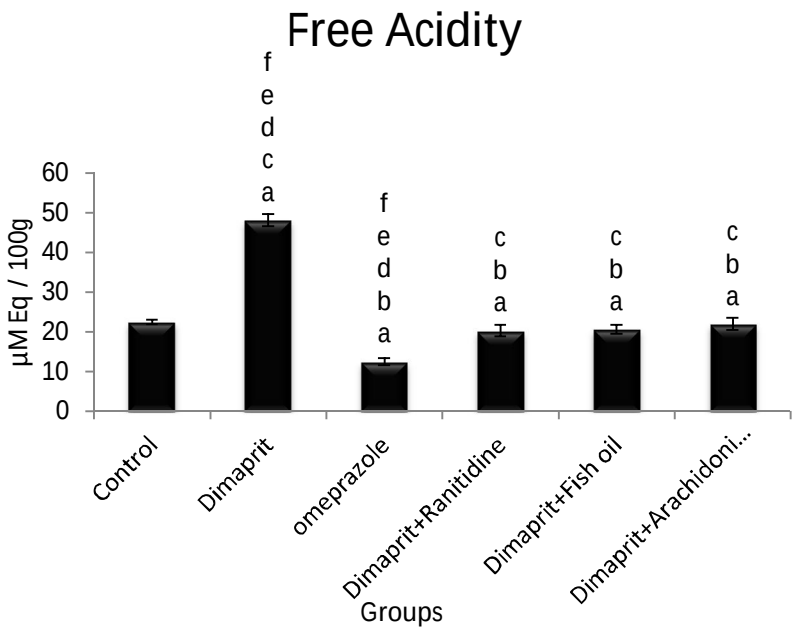
Figure 48: FO and AA on % gastro protection of in Dimaprit (n=6)

Stomach Free acidity:

Free acidity in control was measured at 22.433 ± 0.566. In Dimaprit induced ulcerated control the free acidity was estimated to be 48.2±1.508. Fish oil reduced the free acidity very much around 20.7 ± 1.138. Similarly, Arachidonic acid also reduced the free acidity to 22±1.542. Ranitidine, the standard compound protected the free acidity formation and displayed a free acidity of 20.3 ± 1.478. Omeprazole in untreated control background protected free acidity formation with a free acidity value of 12.5±0.847

Table 38: FO and AA on Free acidity in Dimaprit (n=6)

Group No.	**Treatment (mg/kg)**	**Free fatty acids Mean ± SEM**
Gp –I	Control	22.433 ± 0.566
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	48.2 ± 1.508^{acdef}
Gp-III	Omeprazole 20mg/kg	12.5 ± 0.847^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	20.3 ± 1.478^{abc}
Gp-V	Fish oil (40µl/day.p.o) followed by Dimaprit	20.7 ± 1.138^{abc}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	22 ± 1.542^{abc}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b-Dimaprit vs. O; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others bar.

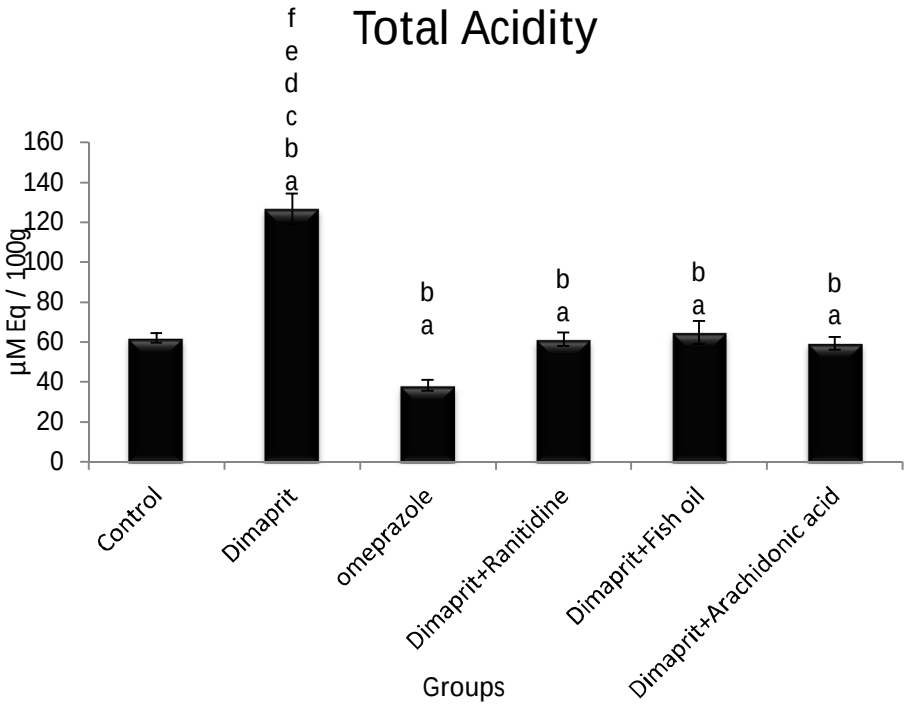
Figure 49: FO and AA on Free acidity in Dimaprit (n=6)

Total acidity:

Total acidity in control was measured to be around 62 ±2.394 and Dimaprit induced ulcerated control the total acidity was measured to be around 126.8±7.752, more than two fold increases. Fish oil prevented total acidity formation with a value of 64.8±5.638. Arachidonic acid was much effective in controlling the total acidity with a value much nearer to the control with a value of 59.4±3.233. The standard compound Ranitidine, reduced the total acidity around 61.3±3.361. Omeprazole in a untreated background, reduced the total acidity much less than the control showing a total acidity of around 38.333±2.716.

Table 39: FO and AA on Total acidity in Dimaprit (n=6)

Group No.	Treatment (mg/kg)	Total acidity Mean ± SEM
Gp -I	Control	62 ± 2.394
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	126.8 ± 7.752^{abcdef}
Gp-III	Omeprazole 20mg/kg	38.333 ± 2.716^{ab}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	61.3 ± 3.361^{ab}
Gp-V	Fish oil (40µl/day.p.o) followed by Dimaprit	64.8 ± 5.638^{ab}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	59.4 ± 3.233^{ab}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b-Dimaprit vs. O; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others bar.

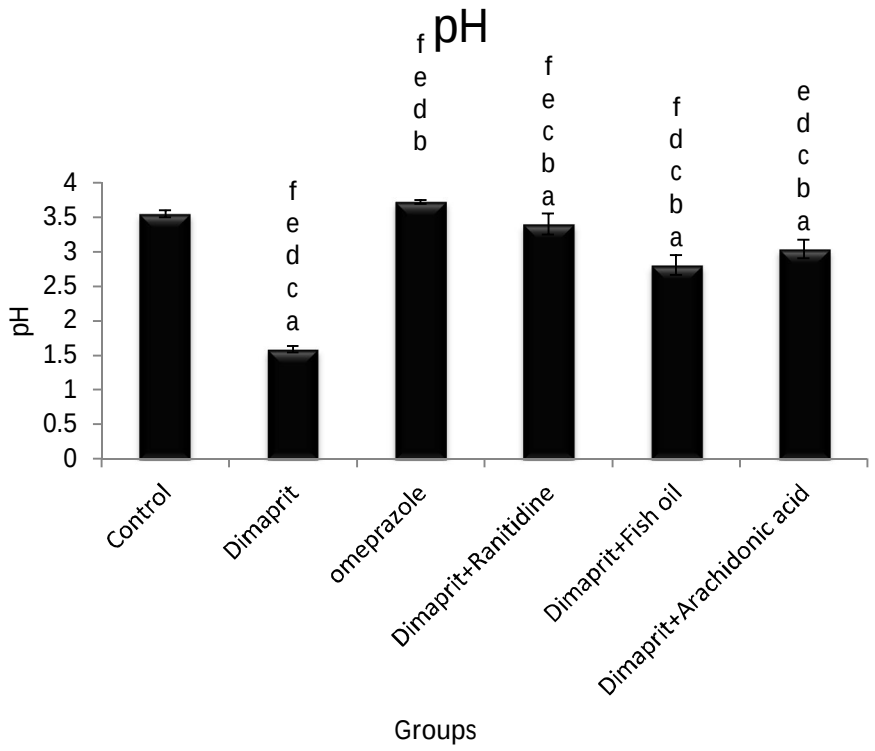
Figure 49: FO and AA on Free acidity in Dimaprit (n=6)

Gastric pH:

Direct measurement of pH is a straight forward method to determine the acidity of gastric juice. The control animals showed a pH of 3.55 ± 0.049. Dimaprit induced ulcerated control animals showed a gastric content pH at 1.59±0.046. Most importantly, fish oil could increase the pH of gastric content to 2.811±0.145. Similarly, Arachidonic acid also increased the pH to 3.04±0.138, which is much closer to the control. The standard drug Ranitidine, also reduced the pH to 3.4±0.152. Omeprazole, in the animals with control background also increased the pH to around 3.723±0.030.

Table 40: FO and AA on pH in Dimaprit (n=6)

Group No.	**Treatment (mg/kg)**	**pH Mean ± SEM**
Gp -I	Control	3.55 ± 0.049
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	1.59 ± 0.046^{acdef}
Gp-III	Omeprazole 20mg/kg	3.723 ± 0.030^{bdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	3.4±0.152abcef
Gp-V	Fish oil (40µl/day.p.o) followed by Dimaprit	2.811 ± 0.145^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	3.04 ± 0.138^{abcde}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b-Dimaprit vs. O; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others bar.

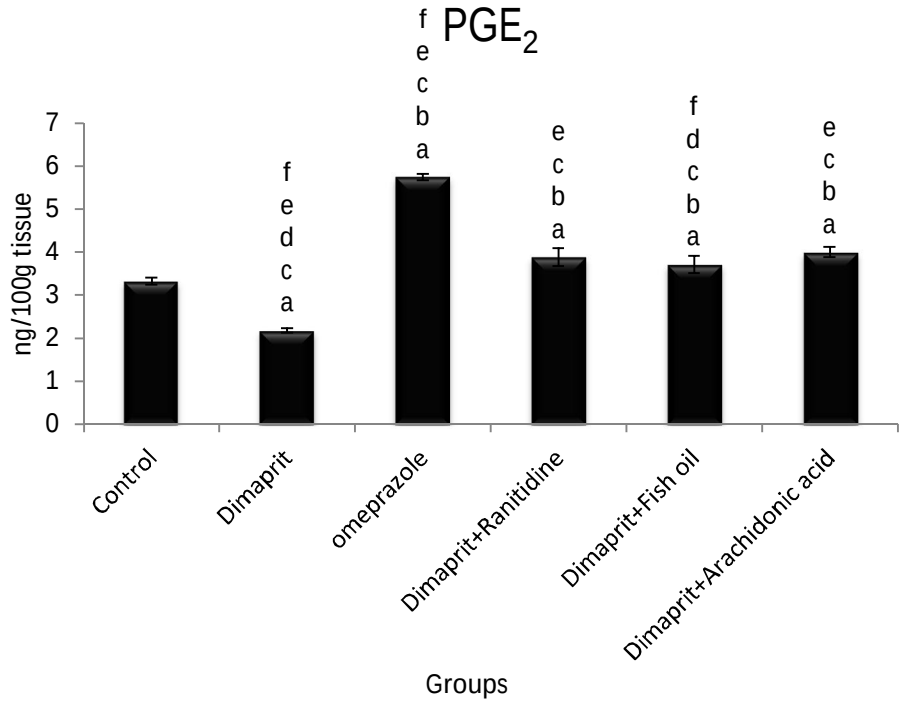
Figure 51*: *FO and AA on pH in Dimaprit (n=6)

Expression of Prostaglandin E_2:

The animals control group showed a range of 3.325± 0.077 for prostaglandin E_2. Upon treatment with Dimaprit to induce gastric ulcer the animals showed a prostaglandin E_2 level of 2.18±0.048. Fish oil induced the expression of prostaglandin E_2 to about 3.72 ± 0.202. Similarly, Arachidonic acid elevated the expression of prostaglandin E_2 to a maximum of 4.004±0.122. The standard drug, Ranitidine also marginally increased the expression to around 3.89±0.209. Omeprazole, increased the expression of prostaglandin E_2 to as high as 5.755±0.075.

Table 41: FO and AA on PGE_2 in Dimaprit (n=6)

Group No.	Treatment (mg/kg)	PGE_2 Mean ± SEM
Gp -I	Control	3.325± 0.077
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	2.18 ± 0.048^{acdef}
Gp-III	Omeprazole 20mg/kg	5.755 ± 0.075^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	3.89 ± 0.209^{abce}
Gp-V	Fish oil 40µl/day.p.o) followed by Dimaprit	3.72 ± 0.202^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	4.004 ± 0.122^{abce}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b-Dimaprit vs. O; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others bar.

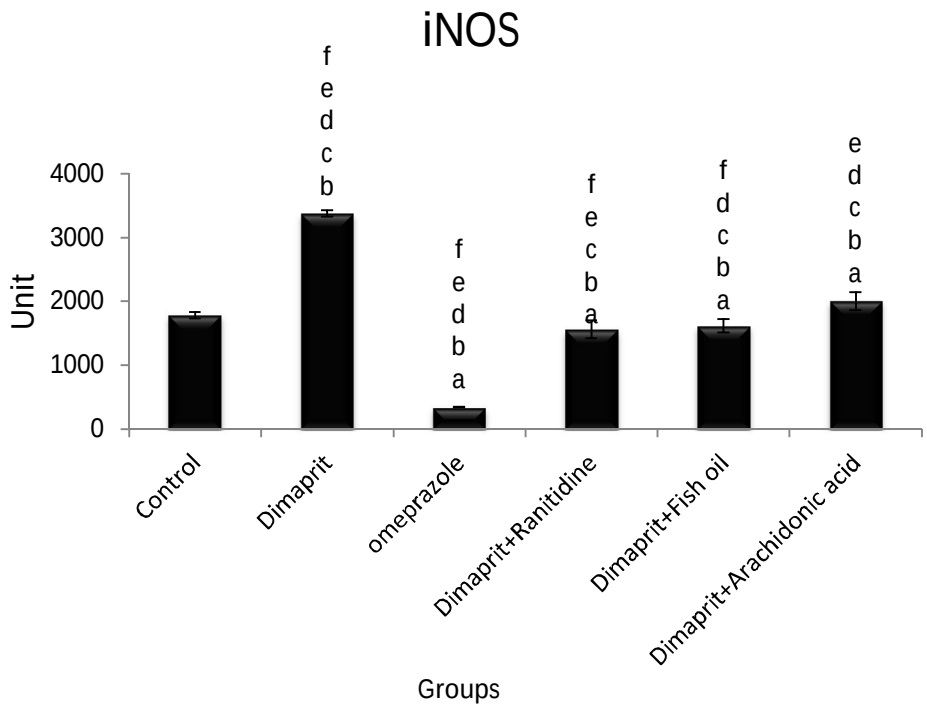
Figure 52: FO and AA on PGE_2 in Dimaprit (n=6)

Expression of iNOS:

Inducible nitric oxide synthase level in control was measured to be around 1784.333± 52.509 and in Dimaprit induced ulcer model the iNOS level was elevated to be at 3379±52.376. Fish oil effectively controlled the expression of iNOS to around 1615±103.64. Similarly, Arachidonic acid also reduced the expression of iNOS with a value of 2008±141.751. The standard drug Ranitidine, effectively reduced the expression of iNOS to around 1563±142.237. Omeprazole, by contrast, reduced the expression of iNOS to a minimum of 339.5±5.303, which is much lower than the control itself.

Table 42: FO and AA on iNOS in Dimaprit (n=6)

Group No.	**Treatment (mg/kg)**	**iNOS Mean ± SEM**
Gp -I	Control	1784.333± 52.509
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	3379 ± 52.376^{bcdef}
Gp-III	Omeprazole 20mg/kg	339.5 ± 5.303^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	1563 ± 142.237^{abcef}
Gp-V	Fish oil (40µl/day.p.o) followed by Dimaprit	1615 ± 103.64^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	2008 ± 141.751^{abcde}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b-Dimaprit vs. O; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others bar.

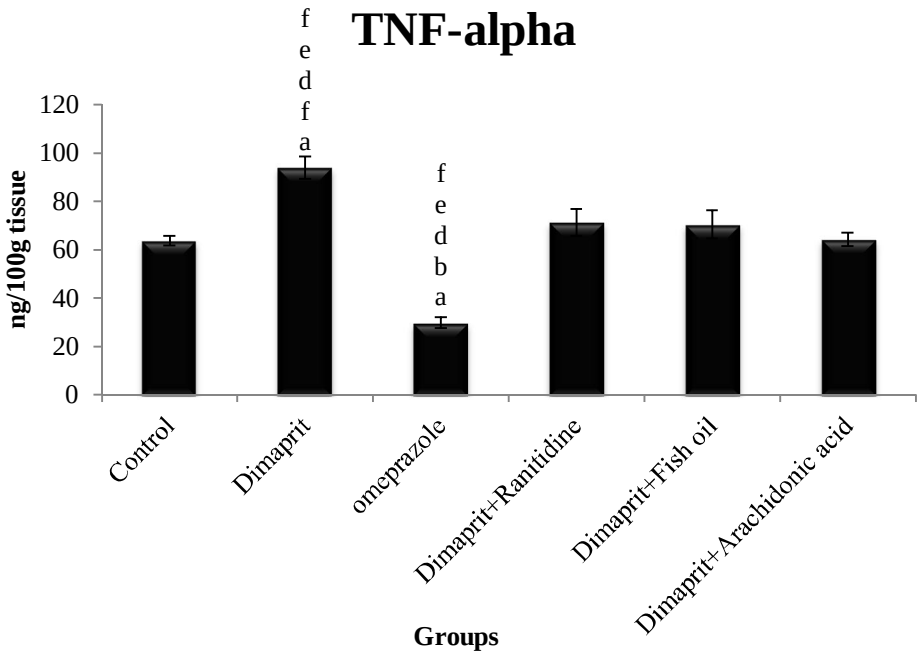
Figure 53: FO and AA on iNOS in Dimaprit (n=6)

Expression of TNF-α:

Tumor necrosis factor-α level in control was around 63.667± 1.994 whereas, ulcerated control using Dimaprit elevated the level of TNF-α to around 94 ±4.695. Fish oil decreased the expression of TNF-α with a value of 70.46 ± 5.793 and Arachidonic acid also reduced the expression of TNF-α to around 64.3 ±2.774 which is much nearer to the level observed in control. The standard drug Ranitidine could only reduce the expression of TNF-α to 71.4 ±5.576. Omeprazole in a control background further reduced the expression of TNF-α to a level of 29.833 ± 2.197 which is extremely lower than control.

Table 43: FO and AA on TNF-alpha in Dimaprit (n=6)

Group No.	Treatment (mg/kg)	TNF-alpha Mean ± SEM
Gp -I	Control	63.667± 1.994
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	94 ±4.695^{afdef}
Gp-III	Omeprazole 20mg/kg	29.833 ± 2.197^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	71.4 ±5.576
Gp-V	Fish oil (40µl/day.p.o) followed by Dimaprit	70.46 ± 5.793
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	64.3 ±2.774



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b-Dimaprit vs. O; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others bar.

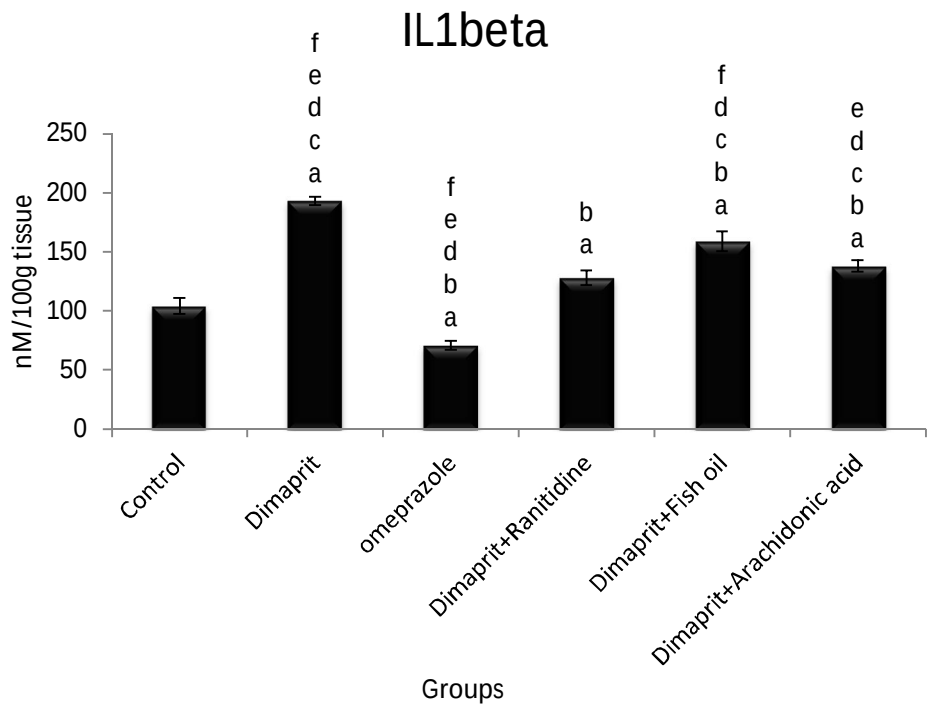
Figure 54: FO and AA on TNF-alpha in Dimaprit (n=6)

Expression of IL-1β:

IL-1β level in control was around 104.167± 6.730 whereas Dimaprit-induced ulcerated control elevated the level to around 193±3.596. Fish oil reduced the IL-1β level to around 159±8.359 and Arachidonic acid also reduced the expression of IL-1β to around 138±5.029. Ranitidine, the standard compound also reduced the level of IL-1β to 128±6.042. Omeprazole in a control background further reduced the expression of IL-1β to 71±3.697.

Table 44: FO and AA on IL1-beta in Dimaprit (n=6)

Group No.	**Treatment (mg/kg)**	**IL1beta Mean ± SEM**
Gp -I	Control	104.167± 6.730
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	193 ± 3.596^{acdef}
Gp-III	Omeprazole 20mg/kg	71 ± 3.697^{abdef}
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	128 ± 6.042^{ab}
Gp-V	Fish oil (40µl/day.p.o) followed by Dimaprit	159 ± 8.359^{abcdf}
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	138 ± 5.029^{abcde}



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b-Dimaprit vs. O; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others bar.

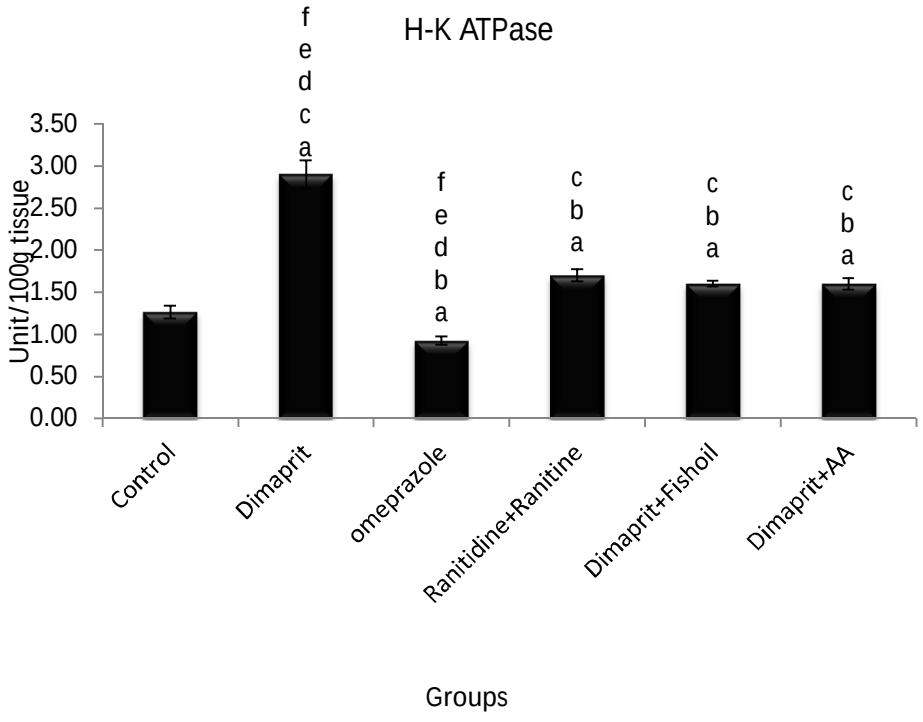
Figure 55: FO and AA on IL1-beta in Dimaprit (n=6)

H^+/K^+ ATPase proton pump:

The H^+/K^+ ATPase proton pump level in control was near to 0.120±0.007 and Dimaprit-mediated ulcerated control showed the level of proton pump to an extent of 1.312±0.042. Fish oil protected the expression of proton pump with a level of 0.302±0.009 and similarly, arachidonic acid also slightly elevated the expression of proton pump to 0.254±0.012. Ranitidine, the standard drug, also reduced the proton pump expression level to 0.25±0.010 and Omeprazole reduced the expression of the proton pump to 0.013±0.001.

Table 44: FO and AA on H+K+ATPase in Dimaprit (n=6)

Group No.	Treatment (mg/kg)	H+K+ATPase Mean ± SEM
Gp –I	Control	0.120±0.007
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	1.312±0.042
Gp-III	Omeprazole 20mg/kg	0.013±0.001
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	0.25±0.010
Gp-V	Fish oil (40µl/day.p.o) followed by Dimaprit	0.302±0.009
Gp-VI	Arachidonic acid (40µl/day.p.o) followed by Dimaprit	0.254±0.012



Mean ± SEM(n=6). The SS of the data is p<0.05. a-Cvs. O; b-Dimaprit vs. O; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others bar.

Figure 56: FO and AA on H+K+ATPase in Dimaprit (n=6)

7. DISCUSSION

The gastric acidity is a consequential donor intended to cause gastric ulceration in the rats. Currently gastric ulcer therapy display modest efficiency in opposition to gastric mucosal lesion/ulceration other than are regularly fraternized by means of more than a few side effects (39) and here it shows in favor of more safer and effective treatment like the nutraceuticals based treatment meant for disorder like to the gastric/peptic ulcer. Therefore the study is based on nutraceutical products which can to resolve peptic ulceration otherwise reducing hyperacidity toward a normal level so that stomach capable of function its physiological position. PUFA is such a mediator that can approach to various view in this observe. Their potency otherwise might or might not be as beneficial as current medications used in increased acidity or else peptic ulceration, but they be able to accept stomach toward function typically which be capable to provide the reason as well as equilibrium be able to be recognized in this view. The production and release of gastric acid is able to add to the prevalence of peptic ulcer disease. There is always a homeostatic equilibrium with acid set free by parietal cell and the bicarbonate and mucus on the opposed side. Added to these are the plethora of enzymes like the pepsin, cholescystokinin and gastrin. Maintaining secretion by a standard stage is the major curative aim. Stress is able to occur as of protracted worry, tension, and emotion, harsh bodily uneasiness, haemorrhage as well as surgical distress, burn and shock, in that way ensuing into brutal gastric ulceration.

At present the investigational *in vivo* model be at idle the most excellent and price efficient method to estimate the effectiveness and strength of new anti-ulcer medications plus their mechanism. In this research study, we have utilised various model of ulcer development i.e. ethanol induced ulcers, go swimming stress induce ulcers, ligation of pylorus induced ulcers, indomethacin plus histamine, as well as Reserpine and Dimaprit induce ulcer. The data obtained in our study suggest with the aim of the PUFA contain oils use in this research i.e. the fish oil and the PUFA comprising Arasco oil were capable to reduce the development of ulcer formation as per the results of the ulcer index and other biochemical parameters.

The results showed decrease inside the acid development as evaluate of through free acidic level. Numerous studies suggest so as to the EtOH-induce gastric lesion be thought to occur since a conclusion of through injure of gastric mucosal cell, ensuing in the progress of free radical as well as increase in the oxidation of lipid (97). Ethanol treated rats demonstrate a major raise in concentration of the gastric hormone in plasma, gastrin along with an raise within the gastric mucosal H+K+ATPase action. The H+K+ATPase enzyme in charge for H+ liberation via the parietal cells. H+K+ATPase are specifically blocked by the drug, Lansoprazole an acidic antagonist utilized to cure ulcer of g.i. tract (98) Commencement of cAMP path stimulate the H+K+ATPase taking place in parietal cell, an elevated capability of proton pump, by means of its incorporation into the apical layer direct to the creation of a secretory canaliculi. In the modern years, the drugs with the intention of decrease the acidic secretion as well as H+K+ATPase reserve contain turn out to be favored curative preference owing to their scientific effectiveness (99). The reserve of H+K+ATPase outcome in the decrease of gastric acidic secretion which

be concordant by means of the current study (100). A correlation of the cAMP and the prostaglandins in response to cholinergic intervention has been studied by several laboratories. It has been therefore reported with the intention of, within rats, acute intragastric management of liberate and Arachidonic acid (AA) and Linoleic acid (LA) is follow by a noticeable raise of gastric prostaglandin defense in opposition to alcohol-induced injury (101).

Pylorus ligation mediated gastric (99) ulcerations are due to acid–pepsin secretion most important to auto incorporation of the gastric mucosa and stop working of mucosal barrier. Several studies (102) encompass exposed with the intention of PUFAs of n-6 series (103) decrease gastric injure in human as well as investigational animals (104).Predominantly these studies encompass been accepted out by means of LA as well as gamma linoleic acid (GLA) derivative from evening primrose oil (EPO). These conclusions are in accordance by means of previous information of the gastroprotective and cytoprotective properties of PUFAs of the n -6 series (105). Our outcomes are also supported by the data concerning the antiinflammatory accomplishment of GLA and its participation in the defensive mechanism of the stomach in resistance to ulceration.

A theory so as to be regularly used to explanation designed for gastric lining damage is to facilitate of an interface among aggressive and self-protective factor in the gut (106). The raise in gastric acid production in the stomachs of pylorus-ligated rats, mucosal injure induce by NSAIDs, vascular damage, haemorrhage and lesion owing to trauma, jointly with a few biochemical proceedings, are the factors

probably capacity to contributing to the process causal for ulcerogenesis, in the current investigational model.

Ulcer formation increase in pyloric-ligated rats is a result from the caustic effects of accumulated gastric acid (107). It is clear from recent studies that EPO given orally to rat's appreciably decreased gastric secretory amount, and free and total acid production in pylorus-ligated rats. These results are in acceptance with previous human and investigational studies representing gastric antisecretory action of LA (104). The anti-secretory action of LA has been credited to the stimulatory result of PGs derivative from n-6 fatty acids (108). It is consequently speculated that the anti-secretory action of EPO might report for antiulcer activity in different experimental models used in this research study, where gastric secretion is concerned in the pathogenesis of gastric ulcers.

These results showing that PUFA considerably confined gastric mucosa in opposition to stress-mediated lesions. Interference of the gastric mucosal blood circulation (109), changes inside gastric secretion (109) as well as gastric motility and trimming of the gastric mucus layer (110) have been consider as pathogenic mechanisms responsible for stress-induced gastric mucosal lesions. The opportunity of PUFA mitigating the ischemic state induced by stress remains open for more investigation. However, n-3 fatty acids have been shown to protect against circulatory disturbance in rat gastrointestinal tract (111).

A profuse quantity of mucus is released from the gastric gland for the period of superficial mucosal injury and offers a good microenvironment in restoration by compensation. Therefore, evaluation of mucin release is important for the learning of mucosal protective mechanisms in conflict to ulcer causing agents. Whilst, increment in gastric acid secretion as well as reduction in mucosal blood circulation and composition of the mucus (109) is the reason for the formation of ulcers in case of water immersion stress test (110). It reduces mucin, surface active phospholipids bicarbonate releases, mucosal production and similarly causes harm by liberation of free radicals.

FO has substantial ulcer protecting roles in various investigational animal models, whose aetio-pathogenesis of ulceration is dissimilar. Nutritional intake of n-6 fatty acid loaded with LA has been established to effect the functional actions of different blood constituents, inducing an inhibitory effect on adhesion of leucocyte, platelet count, aggregation of platelets and collagen deposition. From such reports it can be recommended that the cyto-protective roles of the n-6 fatty acids in the recent study may possibly also be attributed to the alteration of adhesion of leucocytes (112). Finally, the study has revealed that PUFA has a considerable anti-ulcer and cytoprotection on different experimentally produced gastric lesions.

For that reason in the current study the PUFA containing oils guarded although to a smaller degree than commercially accessible medications. We too examined certain preliminary effects of omeprazole on the superoxide dismutase activity and the previous results show reasonable raise in the activity of the enzyme and this is a fine marker to its method in ulcer healing when free radical scavenger systems are used. Dietary intake of n-3 PUFAs improves the healing of colonic

anastomoses and also the colonic injury recovery in a rat model. To conclude, n-3 PUFAs facilitates regeneration and re-epithelialization by inducing quicker recovery of inflammation within the wound milieu. A small randomized clinical trial examined a formula enhanced with FO for patients suffering from pressure ulcers and also reduced development of pressure ulcers in those patients who were getting fish oil as a dietary intake. There are increasing facts that the varied biological roles of n-3 PUFAs add to their renewing actions in antagonism to chronic inflammatory disease. This could efficiently assist to cure the inflammation and induce a conversion from the inflammatory to the proliferative and restoration stages of wound healing n-3 PUFAs can be included into membrane phospholipids that could lead to decreased membrane fluidity. It could be linked with lipid raft association and operate. Lipid rafts are cholesterol-rich micro domains at the host cell surface and are essential for NF-κB-dependent responses to H.pylori. Our studies conducted with the indomethacin and histamine induced injection technique suggest the progress of duodenal lesions that are induced by indomethacin along with histamine in rats is due to elevate in gastric acid secretion as well as due to the impairment of acid-induced duodenal HCO_3^- secretion. This recently recognized model will be helpful for screening antiulcer agents and studying the pathogenesis of duodenal ulcers. Oral administration of Ranitidine was able to greatly decrease the ulcer formation (68).

In present times, a number of studies have recommended that n-3 PUFAs can be transformed into bioactive mediators, comprising resolving, which employ inflammation-resolving functions via counter regulation of lipid mediators containing pro-inflammatory leukotriene (LTs) and prostaglandin (PGs). As a result, a few researchers have examined a long-term therapy of n-3 PUFAs in animals infected with

H. pylori and established that the n-3 PUFAs administration weakened H. pylori-induced gastric inflammation and atrophic gastritis (113). It also decreased the occurrence of H. pylori related gastric carcinogenesis. This may be the early group to manuscript the revitalizing roles of n-3 PUFAs on H. pylori-mediated atrophic change inside stomach (114).Whereas the usage of n-3 PUFAs for management of H.pylori generated GI disorder is quickly affecting keen on medical setting by way of additional studies explanation of the mechanism of action, systematic randomized controlled trials are mandatory toward search out strong proof for the merger n-3 PUFAs into the beneficial armamentarium in close to prospect.

Cyclooxygenase, a prime enzyme liable for PG production, exist in the form of COX-1 and COX-2 has begin to contribute key position in ulcer curative, such so as to its supression leading to important impairments of ulcer curative and angiogenesis. Neutrophils include occupied as in concert key role in opening of endothelial damage. NSAIDS administration shows fast and considerable raise in the number of neutrophils adhering to the vascular endothelium. The adherence of the neutrophils to the vascular endothelium depends upon the β2 integrins as well as interacellular bond particle I (CAM-1) resting on the vascular endothelium. Leukotrines are the group of mediators that aptitude to donate the raise in neutrophil adhesion to the vascular endothelium and the mucosal injury that befall after NSAIDS management. Leukotrines are derivative obtained from arachidonic acid.

EPO, also known as the evening prime rose oil contain n-6 series PUFA and addition to an herbal product is able orally in opposition to gastric secretion and gastric injury in different investigational models. The outcome of several studies demonstrate with the intention of EPO stops a raise in acid release in pylorus ligated

rats and inhibit the development of gastric ulcers make by different ulcer causing drugs, by cytodestructive agents and by anxiety caused by hypothermic limitation. Numerous studies (115) have exposed that EPO or PUFAs of n-6 series decrease gastric injure in humans (104) and experimental animals (102). Biochemical actions are the reasons probably causative to the process original of ulcerogenesis, in the current experimental models. It is based on our result, considered that the anti-secretory action of PUFA might regard for antiulcer activity in different experimental models accustomed in the instant research work, where gastric secretion is concerned in the pathogenesis of gastric ulcers. These outcomes have showing so as to PUFA containing oils give modest gastrointestinal defense within all the induced ulcer models. Thus it can be proficient so as to PUFA containing oils alike the Fish oil and Arasco oil contain antiulcer functions and the mechanisms concerned in these events are being examined. Epidermal growth factor has a major responsibility to participate in gastric mucosal defense. It encourages the discharge of the enzyme cPLA2 and as well the activity of the enzyme cyclooxygenase. It is known that a number of the actions of EGF engross PUFAs. It is possible that its ulcer curative capacity may also be mediated by PUFAs. Improve in the membrane meditation of PUFAs like AA, EPA, DHA is identified to augment the membrane flexibility (116) enhance in the number of insulin receptor with the similarity of insulin to its receptor(122). It is likely to the use of PUFAs might have a like effect on EGF receptor also, which can lead to improved gastric mucosal ulcer curing. Additional, numerous long chain fatty acids are considered to have growth inhibitory action on Helicobacter pylori (117).

Based on, (118) these results it is proposed that the decline in the prevalence of duodenal ulcer may be due to the increased ingestion of vegetable oils rich in long chain polyunsaturated fatty acids. They involve that high intake of PUFAs leading to an augment in the synthesis of PGs which in revolve can improve the gastric mucosal confrontation against ulceration. It has additionally been suggested that PUFAs themselves may keep the gastric mucosa against the injurious events of ulcerogens lacking the need for their change to PGs.

Many previous studies (117) recommended that a fall in the levels of n-6 fatty acids know how to result in a decline in the synthesis and liberate of prostaglandins of the 2 series (119). Since, PGs of the 2 series have gastric mucosal protective actions; decreased levels of n-6 fatty acids may create gastric mucosa more vulnerable to the action of ulcer gens. It is as well likely that PUFAs might have a direct function in the curative of the duodenal ulcer and that their conversion to PGs may not be essential to bring about their protective role against peptic ulcer disease since the results of the present study showed that healing of the ulcer with lansoprazole is associated with normalization of EFA metabolism. This is supported further by the results of (120) who showed that GLA decreases the occurrence of gastric ulceration in aspirin treated rats. The beneficial action of GLA cannot be attributed to PG synthesis since aspirin is a potent PG synthesis inhibitor. In addition, in a recent study (121) we showed that EPA/DHA supplementation is as good as that of an H_2-blocker in healing the duodenal ulcer.

It is reported that Reserpine increased acid secretion in rats (122), which was a possible mechanism leading to mucosal lesions. However, the exact mechanism is not yet clear. Reserpine was thought to exhaust the monoamines at the ends of sympathetic nerves, leading to over activity of the vagal nervous system at the peripheral level. This may lead to an over secretion of gastric acid. In humans, apart from *H.Pylori* and NSAIDs and alcohol it is stress which be able to occur as of protracted anxiety, tension, and emotion, brutal bodily uneasiness, haemorrhage as well as surgical shock, burns and trauma, thus consequential in harsh gastric/peptic ulceration. The gastric acid is an extremely vital provider for the source of ulceration in experimentally produce animal models. Present gastric ulcer therapy demonstrates modest efficiency in opposition to gastric mucosal lesions/ulceration though there is also linked with many side effects and there are continually looking out for extra nutraceuticals centered treatment for gastric ulcer conditions as gastric/peptic ulcers. Hence the studies are being conducted on natural products which are capable of also resolve peptic ulceration or decrease hyperacidity to a normal level so with the aim of stomach could performs its biological role. PUFA are such products that can arise to some possibility in this respect. They might or might not be as effective as contemporary drugs used in hyperacidity or peptic ulceration, still they could permit stomach to perform usually which can aid the purpose and stability can be well-known in this respect. The release of gastric acid can intensify the occurrence of peptic ulcer disease.

Maintaining adequate acid release at a usual quantity is the key medicinal aim of every antacid treatment. Presently the experimental *in vivo* models yet are the greatest and price effective method to assess the efficiency and power of

new anti-ulcer drugs and their mechanisms. One of the most important enzyme mediating acid formations is the H,+K+ATPase. The H,+K+ATPase is the dimeric enzyme accountable for H+ release by the gastric parietal cells. H,+K+ATPase is selectively obstructed by the action of Lansoprazole, an acid release blocker utilized for treating gastric ulcers. Stimulation of cAMP pathway accelerates the H,+K+ATPase on parietal cells, a high functioning proton pump, with its addition into the apical membrane advances to the growth of a secretory canaliculi. In the past years, the treatments that decrease the acid release and causes H+K+ATPase antagonism have grown into ideal therapeutic selection due to their medical effectiveness. The antagonism of H+K+ATPase consequences in the decrease of gastric acid release which is similar with the result s of the current study. In this study, we used reserpine induced model of ulcer formation (122). Reserpine is an anti-hypertensive drug which causes catecholamine depletion. Reserpine was believed to exhaust the monoamines at the ends of sympathetic nerves, leading to over activity of the vagal nervous system at the peripheral level. This can lead to an over secretion of gastric acid. Reserpine (123) is also used to prevent to produce free redicals and reduce the prostaglandin synthesis (124). It has been suggested that peripheral cholinergic and adrenergic mechanisms are used in the ulceration induced by reserpine (125). PUFAs (especially ALA, DGLA, EPA, and DHA), LXs and resolvins suppress IL-1, IL-2, IL-6, and TNF-α production by T cells (126) and therefore work as endogenous anti- inflammatory molecules (127). Though, no studies have shown direct effects of AA on the production of various cytokines, it is commonly accepted that PGF2a, PGE2, LTs and TXA2 derived from AA have modulatory role on production IL-6 and TNF-α. For example, mast cell IL-6

synthesis was stimulate by PGE1 (derived from DGLA) and PGE2 (derived from AA) to a same level of that analyzed in antiIgE-activated cells, whereas the formation of TNF-α was blocked by PGE_1 and PGE_2, not by PGD_2 (128). It was established that PGF2α stimulates IL-6 synthesis via creation of protein kinase C in osteoblast-like MC3T3-E1 cells, and that PGE1 stimulates the synthesis of IL-6 through activation of protein kinase A (129). By keeping in mind the different effects of PGE_1, PGE_2 and $PGF_2\alpha$ on the production of TNF-α and IL-6, the local levels of TNF-α and IL-6 at the sites o injury and swelling may be dependent on the balance between DGLA and AA and the relevant PG products formed from them. It's been experimented that PGE_1 and $PGF_1\alpha$ (derived from DGLA) and TXB2 (derived from AA) block, whereas DGLA and AA per se do not show enough impact on the development of human lymphocytes in vitro at the doses tested. Whereas, PGE_1, PGE_2, $PGF_2\alpha$, and TXB2 suppresses IL-2 formation, and PGE_2, $PGF_2\alpha$, and TXB_2 improved IL-4 synthesis, whereas PGE_2, $PGF_2\alpha$, TXB_2, PGI_2, and $PGF_1\alpha$ increased TNF-α synthesis with not any action on IL-6 synthesis in human lymphocytes in vitro (130). On the other hand, DGLA and AA enhanced, whereas EPA been decreased, the synthesis of IL-4 in human lymphocytes in vitro with not any action on IL-6 production, and are altered by the doses of fatty acids used (130-131). DHA has been proven to suppress IL-1β and TNF-α formation by stimulation of human retinal vascular endothelial cells (132). It's been evident to recommend some of the suppressive actions of EPA and DHA on the formation of pro-inflammatory cytokines and their anti-inflammatory action seem to be intervened by their capability to increase both the PPAR-γ mRNA and protein activity. These results recommend that different PUFAs and their products are different, and at

times entirely opposite, events on the production of various cytokines. Hence, the local concentrations of various PUFAs and eicosanoids formed and the equilibrium in between these different modulators will lastly determine the concentrations and types of cytokines produced and the degree of inflammation.

The information presented here provided scientific evidence that production of TNF- increases the risk of gastric ulcer. Suppression of antisecretory activity, as observed by the decrease in of TNF- and IL-1 production this may be attributed to increase in total acidity and volume of gastric juice (133). Further, the anti-inflammatory activity of these n3 and n6 containing PUFA containing oils reduced the TNF alpha levels and increased the PGE_2 levels (91). This treatment offers cytoprotection by rising inhibition of TNF-α and neutrophil infiltration in mucus. Thus these PUFAs containing oils ultimately inhibit tissue destruction by reactive oxygen showed good gastro protective anti-ulcerogenic activity species. Prostaglandin E_2 possess antiulcer activity beside the ulceration and an important agent as its inhibition is responsible for complex array of ulcer by aspirin (134). Aspirin a well known NSAID (134) inhibits PGE_2 which has healing mechanism, blocks the synthesis of gastroprotective prostaglandins which are synthesized in the mucosal cells erosions and ulcers in gastroduodenal tracked by cyclooxygenase (COX) enzyme action (135- 136). Prostaglandins are creating to be inhibiting the leukocyte enrollment which could give to the favorable effects of these substances in conditions in which the GI mucosa is inflamed (1). On the other hand, the free radical NO is deleterious for the gastric mucosa. Increase of NO synthesis causes the gastric mucosa more inclined to injury. NO affects recruitment of neutrophils to sites of inflammation. Some studies suggest NO diminishes neutrophil infiltration into the

GI tract mucosa (137 -138). In a study conducted it showed that iNOS activity i.e both enzyme and mRNA increased with increase in ulcer intensity and creation in experimental animals. Thus implicating the iNOS in the formation of ulcers. In this thesis work it was found that iNOS activity was reduced by both the anti-ulcer drugs and the PUFA containing oils. The actions associated to gastroprotective effects of nitric oxide involve the promotion of angiogenesis and the reduction in acid secretion. However the iNOS enzyme has different roles to play as well and the endothelial NO which generates relatively large number of NO under certain pathological conditions (139- 140) contributing to mucosal injury and dysfunction. Several plant products have flavonoids which have inhibitory action on iNOS enzyme (141).

The data acquired from our research proposes that the PUFA consisting oils utilized in this work i.e. the fish oil and Arasco oil the n-6 PUFA consisting Arasco oil were capable to diminish the ulcer development as considered on the basis of ulcer index (142). Nutritional supplementation with n-6 fatty acid full in LA has been produced to affect the biological function of several blood components, yielding an antagonistic effect on adhesion of leucocytes, platelet count, aggregation of platelets and collagen deposition (143). Nutritional supplementation with n-3 PUFAs enhanced colonic anastomoses healing as well. n-3 PUFAs also escalated the colonic injury recovery in a rat model. In reality, n-3 PUFAs may speedy resolve the inflammation within the wound milieu, which proceeds to accelerated restoration and re-epithelialization (115). A small randomized clinical trial examined a formula complemented with fish oil in patients with pressure ulcers and prominent reduced advancement of pressure ulcers in those getting fish oil supplementation. There is

rising proof that the varied natural parts of n-3 PUFAs contribute to their renewing actions against chronic inflammatory disease. This might efficiently aid to resolve the inflammation and stimulate a changeover from the inflammatory to the proliferative and restoration stages of wound healing. Biochemical affairs are the aspects probably backing to the development triggering ulcer formation, in the current experimental models. It is hence guessed that the anti-secretory action of PUFA may responsible for antiulcer activity in several experimental models utilized in the current research, where gastric release is participating in the etiology of gastric ulcers. These outcomes have revealed that PUFA containing oils delivered adequate gastrointestinal defense in each of the stimulated ulcer animal models. Thus it could be presumed that PUFA containing oils alike the Fish oil and Arasco oil have antiulcer functions.

In humans, apart from *H.Pylori* and NSAIDs and alcohol it is stress which could ascend from continued stress, emotional worry and sentiment, rigorous physical distress, bleeding and invasive shock, injuries and pain, thus causing severe gastric/peptic ulceration. The gastric acid is also a fundamental reason for the origin of ulceration in experimentally stimulated animal models. Present gastric ulcer treatments display modest effectiveness against gastric mucosal lesions/ulceration nonetheless is also related with several adverse effects and there is continuously search for more nutraceuticals centered treatment for gastric ulcer complaints like the gastric/peptic ulcers. Therefore the experiments are being conducted on natural yields which could either resolve peptic ulceration or decline hyperacidity to a regular level so that stomach could accomplish its biological and functional roles (144). In current years, increasing interest in alternative therapies can be seen,

especially those from plants and other natural products due to their supposed relatively lesser side effects, ease of accessibility and affordability. Plant medicines with ethno medicinal use in peptic ulcer management needed to be monitored for their effectiveness and likely isolation of lead compounds (103). This necessitates use of suitable animal models of varied ulcers. The inadequate number of antiulcer models for drug improvement against gastric and duodenal ulcer studies has slowed down the development of targeted therapy in this field and PUFA are those products that could reach to certain prospects in this respect. PUFAs are those fatty acids some of which have minimum of two carbon-to-carbon double bonds in a hydrophobic hydrocarbon chain, which typically includes X-Y carbon atoms and terminates in a carboxylic acid group (145). On the other hand, *in vitro* studies show that H_2R agonists mimic the actions of histamine, which hinders the secretion of proinflammatory cytokines and stimulates the creation of anti-inflammatory cytokines in human peripheral blood mononuclear cells. Moreover, the effects induced by histamine were primarily mediated by H_2R, proven by the fact that these effects were blocked by cimetidine. Furthermore, the H_2R mediates suppression of TNF-alpha production by mast cells (146). IL-1beta, is also relevant to the pathogenesis of peptic ulcer disease (147). In an interesting publication, some researchers endeavor to define the role of IL-1 and gastric acid secretion in gastric ulcer recurrence. They utilized a recognized rat model in which antral ulcers, induced by submucosal injection of 20% acetic acid, are known to recur on intra-peritoneal injection of IL-1. They found that round the clock monitoring following IL-1 injection, expression of adhesion molecules and concentrations of IL-1beta and TNF-alpha in scar tissue had increased(148). Our data are reliable with previous

studies showing that oils with PUFA inhibits histamine induced TNF-α production from monocytes and mast cells(149). The data obviously shows the antacid properties of oils with PUFA as well as their famous action on IL-1 beta and TNF-alpha levels. The data presented here provided scientific evidence that production of TNF- increases the risk of gastric ulcer. Suppression of antisecretory activity, as experientianced by the decrease in of TNF- and IL-1 production this may be attributed to total acidity and volume of gastric juice. Further, the anti-inflammatory activity of these n3 and n6 containing PUFA containing oils reduced the TNF alpha levels. This treatment offers cytoprotection by increasing inhibition of TNF-α and neutrophil infiltration in mucus. There are at least two independent families of PUFAs, depending on the parent fatty acid from which they are produced. They include: the "ω- 3" series derived from ALA (18:3,)ω-6derived from *cis*-6).ω-LA (18:2,).

Thus these PUFA containing oils eventually inhibit tissue destruction by reactive oxygen showed good gastro protective antiulcerogenic activity species. Nutritional supplementation with n-3 PUFAs improved colonic anastomoses restoration. n-3 PUFAs enhanced colonic anastomoses healing as well. n-3 PUFAs also escalated the colonic injury recovery in a rat model. In reality, n-3 PUFAs may speedy resolve the inflammation within the wound milieu, which proceeds to accelerated restoration and re-epithelialization (150). A small randomized clinical trial examined a formula complemented with fish oil in patients with pressure ulcers and prominent reduced advancement of pressure ulcers in those getting fish oil supplementation. There is rising proof that the varied natural parts of n-3 PUFAs contribute to their renewing actions against chronic inflammatory disease (151).

This might efficiently aid to resolve the inflammation and stimulate a changeover from the inflammatory to the proliferative and restoration stages of wound healing. Biochemical parameters are the aspects probably backing to the developments triggering ulcer formation, in the current experimental models. It is hence guessed that the anti-secretory action of PUFA may be responsible for antiulcer activity in several experimental models utilized in the current research, where gastric release is participating in the etiology of gastric ulcers. There is need to carry out more comprehensive pre-clinical studies with these oils with PUFA to elucidate their molecular mechanisms in anti-ulcer activity.

8. SUMMARY

The outcomes of this study advise that PUFAs are effective in various forms of gastric ulcers induced by different techniques. Amongst the techniques used were the ethanol induced, pyloric ligation, NSAID induced and Dimaprit and reserpine induced ulcer models. Standard parameters like the Ulcer index, total and free acidity levels and gastric pH were evaluated. The purpose of using several techniques was to study the effects synonymous to the H.Pylori induced ulcers, anatomical alteration, stress and drug induced ulcers in humans. Our study suggests that PUFAs (n-3 and n-6) are effective in reducing gastric erosion and inflammation and levels of acidity. This study also suggests that PUFAs may be acting at multiple biochemical levels and modulating and attenuating the effects of key mediators in the inflammation of the gastric mucosa like the PGE2, Il-1 β and H+K,ATPase, iNOS etc. To compare the effects of the PUFAs some conventional anti ulcer drugs like the Ranitidine and Omeprazole were used in these studies. It is now known that Omega-3 polyunsaturated fatty acids (n-3 PUFAs), generally eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been recognized as essential long-chain fatty acids imposing either optimal health promotion or the rescuing from chronic inflammatory diseases such as atherosclerosis, fatty liver, and various inflammatory gastrointestinal diseases. Omega-3 (n-3) [n-3 PUFAs, eicosapentaenoic acid (EPA 20:5n-3), and docosahexaenoic acid (DHA 22:6n-3) are the long-chain PUFAs, which are essential fatty acids as they can be synthesized by mammals from other dietary precursors containing n-3 PUFAs. They are sufficiently found in fish oil. Fatty acids are key nutrients affecting early growth and

development and preventing chronic disease in later life. A number of animal and human studies have provided convincing evidence for the anti-inflammatory effects of n-3 PUFAs. n-3 PUFAs are beneficial as a dietary supplement in chronic inflammatory conditions like the rheumatoid arthritis by reducing the level of AA-derived eicosanoids and inflammatory cytokines, which include interleukin-1, interleukin-2, interleukin-6, and interleukin-8, as well as TNF-α and LTB4, promoting anti-inflammatory activities. PUFAs are known to restrain the growth of Helicobacter Pylori has been shown to increase the levels of endogenous PUFA metabolism like the n-6 in the gastric mucosa thus suggesting that PUFAs contribute to gastroprotective effect.

Nutraceutical based therapy may be cost effective, with lesser side effects and equieffective in such inflammatory conditions like the gastric ulcers. Moreover these are also endogenously present and it is important that newer drugs can also be designed to affect their levels and reduce the intensity of the disease states. There is need to study the epigenetics of the role of PUFAs and its contribution to physiological processes. This study also highlights the importance of using the naturally occurring PUFAs in conjunction with conventional anti-ulcer drugs.

The impact of this study is to highlight the importance of nutraceutical based therapy for curing gastric ulcers and to examine the role of endogenous fatty acids and the epigenetic factors leading to the disease state. Structure activity relationship studies can be carried out to develop PUFA based agents for therapeutic purpose.

9. CONCLUSION

Gastric or duodenal ulcers develop when stomach lining is exposed to the acids produced in the digestive juices. Furthermore, the vital cause of ulceration is imbalance between gastric offensive factors (pepsin, lipid Peroxidation, nitric oxide) and defensive factors (mucin secretion, glycoprotein and glutathione). Normally there is a balance between HCl and bicarbonate, mucus secretion. Imbalance can result in hyperacidity and ulceration.Numerous factors are implicated that play a fundamental role in the pathogenesis of ulcerations like, sedentary alcohol intake, life style, drugs, spicy food and various bacterial infections like Helicobacter pylori. It has been documented that during ulceration various mediators like nitric oxide (NO), tumor necrosis factor-α (TNF-α), reactive oxygen species (ROS), oxidative stress are implicated in the pathogenesis of ulceration. During gastric and duodenal ulceration there is increased expression of inducible Nitric oxide synthase (iNOS). This study conducted in different animal's models of ulcers provides evidence that PUFA containing oils like the fish oil and Arasco oils are effective as anti-ulcer agents and reduce the ulcer index and free acidity levels apart from their salutary effects on various biochemical mediators of ulcer formation as described in this thesis. It is discretionary to use nutraceutical based therapy and the PUFA oils in the palliative care of chronic gastrointestinal tract mucosal ulcers.

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