SRIWIJAYA JOURNAL OF MEDICINE

Potential of Anti-Secretory and Cytoprotective Effect from Azadiradione as GERD Ulcer Prevention

Corinna Faustina[1], Villicia Angellika[1], Alya Rahmadani[1], Rosalie Dorothea[1], Ricky Fajar Adiputra[1], Ziske Maritska[2]*

[1]Medical Study Program, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[2]Department of Biology Medicine, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
E-mail: ziske_maritska@unsri.ac.id

Abstract

Gastroesophageal Reflux Disease (GERD) is a pathological state due to stomach reflux within the esophagus, which causes necrosis of esophageal mucosa, resulting in erosions, ulcers, and other complications. GERD is still a growing global issue with 783.95 million cases worldwide in 2019. Although PPIs were used as medications to treat GERD, there were refractions and adverse effects to the patients. Azadiradione, terpenoids that can be found in *A. indica* and various plants, has potential as alternative H+/K+ ATPase inhibitor sources with its anti-secretory and cytoprotective activity. Some studies explain that there is also adverse effects of azadiradione. Further research is needed to determine alternative sources, safety dosage, usage duration, side effects, also drug-food or drug-drug interaction of azadiradione.

Keywords: *anti-secretory, Azadiradione, cytoprotective, GERD, prophylaxis, stomach acid, ulcer*

1. Introduction

Gastroesophageal Reflux Disease (GERD) is an uncontrolled condition as a result of stomach reflux toward the esophagus, with diverse symptomps caused by esophagus, pharynx, larynx, and airways involvement.[1] Typical GERD symptoms are retrosternal pain, dysphagia, stomach pain, coughing, wheezing, belching, and regurgitation in seventy percents of patients. There are unsual symptomps that are irrelevant to the gastrointestinal system sych as coughing, laryngitis, recurring pharyngitis, wheezing, and chest pain.[2]

Excessive acid and pepsin reflux causes necrosis of esophageal mucosa, resulting in erosions and ulcers. The duration of stomach acid and bile salt contact often determines the extent of esophageal mucosal injury.[3] Stricture, Barrett's esophagus, pneumonia, and lung fibrosis can all be the complications of GERD.[4]

People's lifestyle, nutrition, medication, and smoking habits have changed dramatically throughout the years, and GERD is still a growing global issue today.[5] In 2019, there were 783.95 million cases of GERD on the worldwide. The overall number of cases grew between 1990 and 2019.[6]

More than 4,000 years ago, Chinese herbalists were believed to have utilized seminal fluid and newborn urine remedies to treat symptoms. In addition, coral powder, kaolin, pearl powder, acid, cannabis, and cocaine were also used later in the hopes of alleviating any symptoms. Antacids were developed as an appropriate clinical intervention that eventually led to the creation of acid inhibitory therapy in the 1970s, known today as H2 receptor blockers. More research and development were performed, as in the 1980s proton pump inhibitors (PPI) were introduced and used in most nations to reduce gastric acid production by inhibiting H^+/K^+ ATPase in the stomach.[4]

Despite the fact that PPIs used as the common treatment for GERD, roughly 20-40% of GERD patients do not react to PPIs.[7] Long-term use of proton pump inhibitors (PPIs) might result in increased hypochlorhydria,

gastric pH, and in some cases achlorhydria linkage to other acid-suppressing medications. It may also result in substantial vitamin (B12 and C) and mineral (iron, calcium, and magnesium) shortages, that require stomach acid for absorption and bioavailability. Pregnant women that use PPIs for long-term can increase the incidence of congenital abnormalities.[8]

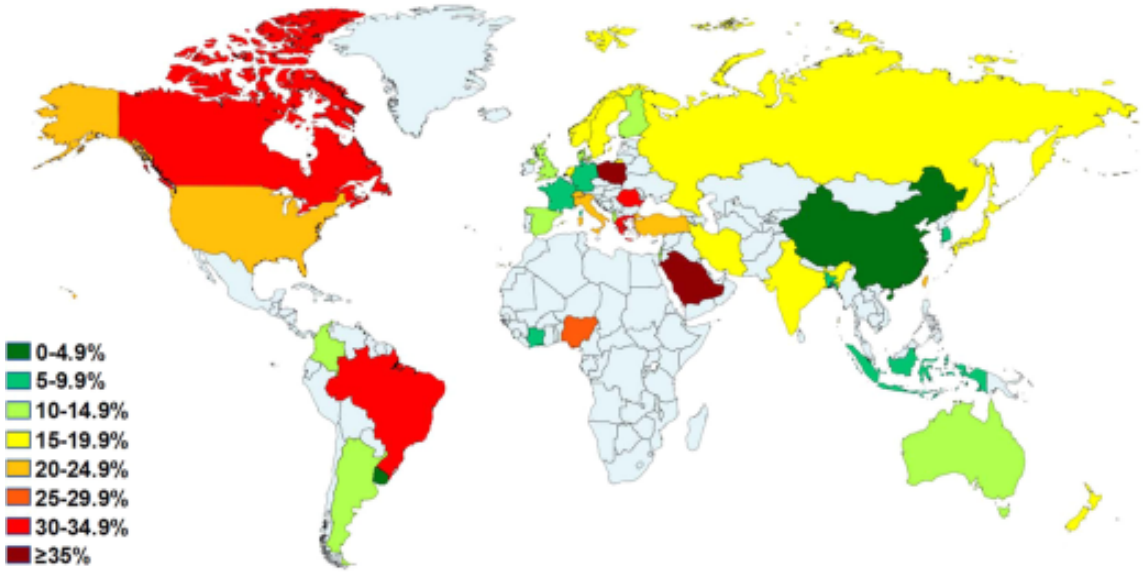

Figure 1: Geographic distribution of GERD prevalence[6]

The refractions and adverse effects of PPIs shows that we need natural gastric H^+/K^+ ATPase inhibitors with nutraceutical value and potential for the prevention of gastric acid-related illnesses. Plant-based proton pump inhibitors have been discovered as a result of research on plants used for food and medicinal.[9] Azadiradione, isolated from ethanolic extracts of *A. indica* seeds, displayed strong antiulcer action by inhibiting H^+/K^+ ATPase via its cytoprotective and antisecretory effect.[10] This review aims to present an overview of the azadiradione and its potential as GERD ulcer prevention.

2. GERD Ulcer

Gastric ulcer is a condition where a defect or lesion can be found in gastric mucosa or even extends into deeper layer of gastric wall.[11] Gastric ulcer (GU), the most common diseases, that have prevalence of 20–60 per 100,000 people and a mortality rate 5–10% worldwide. Higher risk of stomach cancer can be induced by GU or duodenal ulcer when the occurrence is longterm.[12]

NSAID, including aspirin, is a drug that is widely used as an analgesic, antipyretic and anti-inflammatory. The continuous use of NSAIDs, a non-selective drug, may induced gastric ulcer by the mechanisms of inhibition of the enzyme cyclo-oxygenase (COX). The production of cytoprotective prostaglandins (PGE2 and PGI2) consequently decreased when both COX1 and COX 2 inhibited, which triggering the production of mucus and bicarbonate from the gastric mucous cell and diminish the production of gastric acid from the parietal cell. As a result, it decreases the cytoprotective agents (mucus and bicarbonate) and increases the harmful agent (gastric acid and causes higher risk of gastric ulcer.[13]

Alcohol (ethanol) is another ulcerogenic substance that causes gastric ulcers. The mucosal layer of the stomach is affected and exposed to pepsin's and hydrochloric acid's proteolytic and hydrolytic effects. Ethanol directly affects and damages the parietal cells. Reactive oxygen species (ROS) and pro-inflammatory cytokines are produced in greater amounts, blood flow is decreased, and microvascular damage lowers cellular antioxidant levels.[14] Additionally, alcohol-induced mucosal damage can increase gut permeability, which allows bacterial endotoxins that are physiologically incapable to cross the intestinal wall to enter the blood or lymph.[15] All of this mechanism then leads to the erosions of gastric mucosa and higher the risk of getting gastric ulcer.

3. Mechanism Of Gastric Acid Secretion

Gastric acid secretion is stimulated by anticipation of food and the presence of undigested food in the stomach and releases acetylcholine (ACh) around G cells and parietal cells. These cells secrete gastrin and HCl in response to ACh receptors on their surface. The bloodstream carries gastrin, which eventually reaches the parietal cells then bounds to its receptor on the outside of parietal cells. The combined action of gastrin and ACh on the parietal cells results in a high flux of HCl. The gastric response to stimuli produced in the brain is called the cranial phase of gastric secretion.[17]

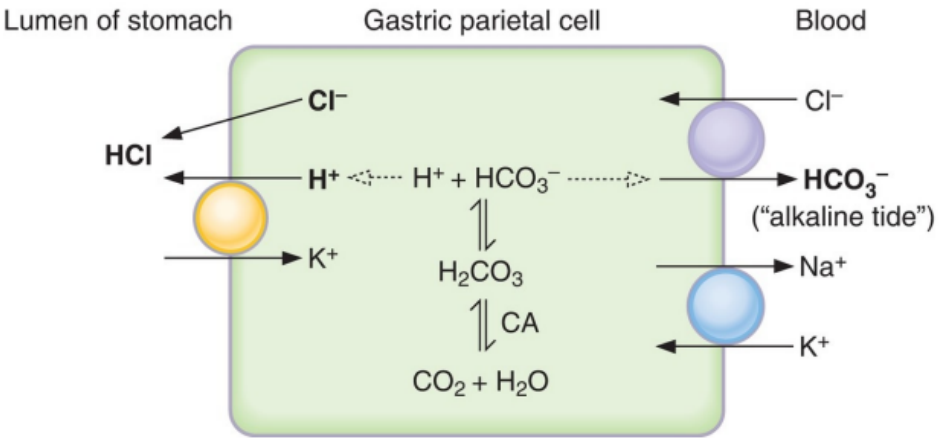

Figure 2: Simplified mechanism of H+ secretion by gastric parietal cells. CA = carbonic anhydrase[16]

The second phase or gastric secretion initiated when food enter the stomach. The buffer role of food removes the inhitibing effect of acid secretion on G-cell, leads to even greater acid secretion by parietal cells.[18]

On the other side, histamine also plays a role as an amplifying substance in gastric acid secretion. Same as gastrin and Ach receptors, histamine receptors are also located outside the cell membrane. When all the three receptors are active, their induction is very strong. Histamine is secreted by mast cells and enterochromaffin-like cells in the parietal mucosa.[19]

Gastrin and ACh induce the release of histamine from the histamine-secreting cells. Thus, by stimulating the release of histamine, gastrin and ACh increase the gastric acid secretion. The pH of the stomach decreases as gastric secretion and digesting continue. When the stomach's pH drops to 2, gastrin secretion is reduced, and at a pH of 1, it is

entirely stopped. As a result, there is no longer gastrin stimulation to the parietal cells, and acid secretion is decreased.[19]

Gastric acid production is also affected by the enteric conditions. The movement of low-pH substances from the stomach toward the intestine lowers its pH, preventing gastric acid secretion. Secretin, a hormone secreted in the duodenum, and enteric nervous system neural reflexes could be involved. Pepsinogen secretion seems to be regulated similarly to HCl secretion. Even so, the regulation of pepsin efflux has received far less attention than the regulation of HCl production[16].

4. The Action of PPIs

PPIs are a type of medication that precisely targets the H+/K+-ATPase enzyme present in stomach parietal cells. Gastric acid-related illnesses such as erosive esophagitis, GERD, and PUD are commonly treated by PPIs. Proton pump inhibitor can also be used to treat variety of conditions caused by gastric acid, including cancer, *H. pylori* infection, viral diseases, respiratory problems, etc. Omeprazole, Esomeprazole, Lansoprazole, Dexlansoprazole, Pantoprazole, and Rabeprazole are some of the commercially available PPIs.

Proton pump inhibitors (PPIs) block the parietal cell's hydrogen pump directly and without triggering any membrane receptors[20]. When PPI enters the body, absorbed by the body, and distributed through circulation, it acts on the parietal cells of the stomach, where acid secretory canaliculi present. At <5 pH, PPI binds covalently to the cysteine group on the H^+/K^+-ATPase. As a result, it inhibits the exchange of H ions and K ions, causing inhibition of H-Cl bound and acid secretion in gastric lumen.[21]

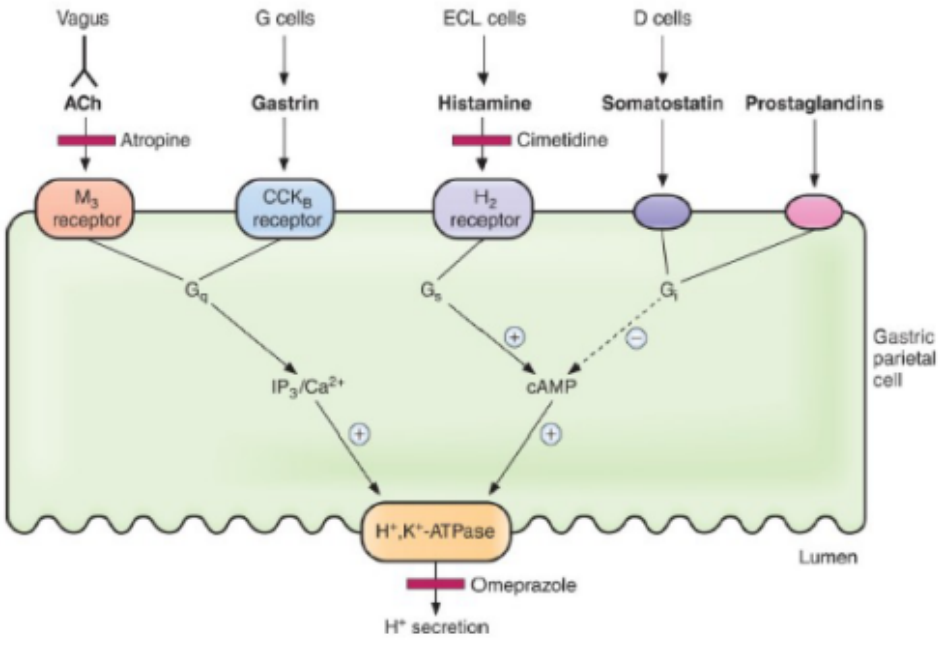

Figure 3: PPI (Omeprazole) inhibits the H^+/K^+-ATPase and reduces the H^+ secretion[16]

5. PPIs Adverse Effects

In patients with GERD and in NSAID induced gastric ulcer, PPIs are frequently administered for short-term healing and maintenance of healing, then given PPIs at higher dosages for up to months than usual for symptom control[22]. PPIs have an excellent safety profile and well tolerated. These drugs become the most frequently prescribed medications in both main and specialized

care. But, along with the increased long-term use of PPI, in 2010 and 2011, the FDA issued a safety alert regarding a potential increase in the risk of fractures related to osteoporosis and low level of magnesium in the body[23].

The most common adverse effects of short-term PPIs usage include nausea, vomiting, diarrhea, and stomach pain. These side effects are minor and go away after the medication is stopped. Long-term PPI use has been linked to a variety of unusual and serious side effects, including vitamin B12 deficiency, hypomagnesemia, iron deficiency, RAHS, osteoporosis-related fractures, tubulointerstitial nephritis, chronic kidney disease, pneumonia infection, *Clostridium difficile* infection, rhabdomyolysis, anemia, and thrombocytopenia[23].

Srinutta et al, in their recent systematic review, shown association of the low-dose PPI use with hypomagensemia compared to non-PPI use. With increasing dose of PPI use, there's increasing risk of hypomagnesemia. However, the mechanism of PPI causing hypomagnesemia is still a subject for future research.[24]

Numerous clinical studies show clear association of PPI therapy with increased risk of osteoporosis-related fractures. According to a recent big meta-analysis study, PPI use is significantly linked to an elevated risk of hip fracture[25]. As the gastric acid is essential for calcium absorption, osteopotosis is believed to be caused by decreased calcium absorption, possibly as a result of the reduction of stomach acid (hypochlorhydria) by PPIs, which is reduce calcium's solubility and cause malabsorption, and it worsens loss of bone mineral density from osteoclast activation and bone resorption.[26,27]

PPI has been linked with cardiovascular disease in recent studies. PPIs inhibits dimethylarginine dimethylaminohydrolase (DDAH) activities. DDAH is responsible for metabolizing asymmetrical dimethylarginine (ADMA), a competitive inhibitor of nitric oxide (NO) synthase. In both mouse and human models, PPIs elevates plasma ADMA levels which resulted in reduction of DDAH activites and NO levels. NO have vasoprotective property as it decreases platelet interactions with vascular endothelium. Reduced level of NO cause by PPI can lead to cardiovascular impairment and resulting in the increase of incidence of cardiovascular disease related morbidity.[28]

6. Anti-Secretory and Cytoprotective Effect from Azadiradione

Azadiradione is a tetracyclic triterpenoid that can be found in Azadirachta indica, Cedrela odorata, Lansium domesticum, and various organisms[29]. *A. indica* has been used for many years as traditional medicine. There are several effects of *A. indica*, namely malarial, bacterial, and viral infections; insecticide properties; antifertility; and hypotensivity with minimal negative chronotropic effects.

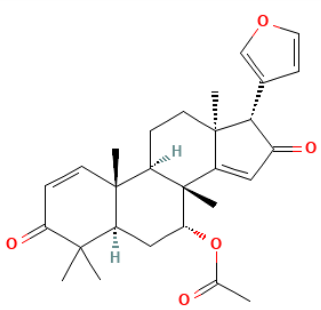

Figure 4: Azadiradione chemical structure[29]

Cytoprotective and antisecretory effect in a cold constraint, aspirin, alcohol, and pyloric ligation induced ulceration being has showing by Azadiradione as a strong antiulcer action by blocking H+/K+ ATPase. Azadiradione from leaf extracts demonstrated effective protection against ulceration by reducing serum levels of alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transpeptidase, alkaline phosphatase, total bilirubin, creatinine, uric acid, and urea in a cisplatin-induced mouse. The study's findings that leaves can protect against hepatotoxic and nephrotoxic consequences.[10]

The uncontrolled release of acid from the parietal cells of the gastric mucosa leads to hypersecretion in the stomach. During ulceration, peptic juice production, reactive oxygen species-induced oxidative damage, and apoptotic cell death were all significant. In order to effectively treat gastroduodenal ulceration, reactive oxygen species must be neutralized, apoptosis must be blocked, and cell proliferation must be promoted.[10]

Aqueous leaf extract, conducted to three groups of Wistar rats at dosages of 150, 300, and 600 mg/kg body weight, significantly decreased the number of ulcers induced on by pyloric ligation, aspirin consumption, and cold restraint stress. Over temperature and time, the total phenolics and vitamin C content of an aqueous crude extract of leaves maintained at room temperature fell significantly; however, ferulic acid levels increased. A. indica-derived compound called nimbidin has demonstrated antisecretory activity in rats and cats with pylorus ligation. The H receptor antagonists' activity was comparable to that of nimbidin in this case.[10]

7. Challenges

Azadiradione produced by neem extract may have adverse effects. A nine-month-old child's symptoms of idiopathic intracranial hypertension (IIH) and the use of Neem leaf extract were found to be related in a study. Neem oil was also revealed to have adverse effects, including ataxia, metabolic acidosis, renal failure, encephalopathy, and refractory seizures.[30] One of the sources of medical information, WebMD, describes a few particularly worrying side effects of taking neem extracts. In conclusion, it considers these extracts to be severely harmful to the liver and kidney due to the lack of studies.[31] Studies have only partially revealed the cytoprotective and antisecretory effects of azadiradione. Therefore, further reserach is required to determine whether azadiradione in other plant extracts has equivalent side effect.

8. Conclusion

Azadiradione has almost the same benefits as PPI class drugs but with unknown adverse side effects. Azadiradione can be obtained from plant extracts, one of which is *A. indica*. However, more investigation is needed to obtain the alternative sources, safety dosage, usage duration, side effects, also drug-food or drug-drug interaction of azadiradione. We hope azadiradione can be an alternative solution for GERD ulcer prevention.

References

1. Sholihah RI. Evaluasi Penggunaan Obat Golongan PPI (Proton Pump Inhibitor Pada Penderita GERD (Gastroesophangeal Reflux Dissease) Rawat Inap di RSUD Kota Madiun. 2019;51(1).
2. Yahya AI. Introductory Chapter: Gastroesophageal Reflux Disease. Gastroesophageal Reflux Disease - Theory and Research. 2019 Apr 3;
3. Chiejina M, Samant H. Esophageal Ulcer. StatPearls; 2022.
4. Boulton KHA, Dettmar PW. A narrative review of the prevalence of

gastroesophageal reflux disease (GERD). Annals of Esophagus. 2021 Mar 1;5(0).
5. Zhang D, Liu S, Li Z, Wang R. Global, regional and national burden of gastroesophageal reflux disease, 1990–2019: update from the GBD 2019 study. Ann Med. 2022 Dec 31;54(1):1372–84.
6. Nirwan JS, Hasan SS, Babar ZUD, Conway BR, Ghori MU. Global Prevalence and Risk Factors of Gastro-oesophageal Reflux Disease (GORD): Systematic Review with Meta-analysis. Sci Rep. 2020 Dec 2;10(1):5814.
7. Azzam RS. ARE THE PERSISTENT SYMPTOMS TO PROTON PUMP INHIBITOR THERAPY DUE TO REFRACTORY GASTROESOPHAGEAL REFLUX DISEASE OR TO OTHER DISORDERS? Arq Gastroenterol. 2018 Nov 1;55Suppl 1(Suppl 1):85–91.
8. Koyyada A. Long-term use of proton pump inhibitors as a risk factor for various adverse manifestations. Therapie. 2021 Jan 1;76(1):13–21.
9. Adinortey MB, N'guessan BB. H+/K+-ATPase Inhibitors from Plants: A Potential Source for Drug Discovery. Natural Products as Enzyme Inhibitors. 2022;125–36.
10. Saleem S, Muhammad G, Hussain MA, Bukhari SNA. A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of Azadirachta indica. Phytother Res. 2018 Jul 1;32(7):1241–72.
11. Lanas A, Chan FKL. Peptic ulcer disease. Lancet. 2017 Aug 5;390(10094):613–24.
12. Rahman Z, Dwivedi DK, Jena GB. Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-1 signalling pathway. Hum Exp Toxicol. 2020 Apr 1;39(4):547–62.
13. Serafim CA de L, Araruna MEC, Alves Júnior EB, Silva LMO, Silva AO, da Silva MS, et al. (-)-Carveol Prevents Gastric Ulcers via Cytoprotective, Antioxidant, Antisecretory and Immunoregulatory Mechanisms in Animal Models. Front Pharmacol. 2021 Aug 23;12.
14. Rahman Z, Dwivedi D, Jena G. Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-1 signalling pathway. Hum Exp Toxicol. 2020 Apr 26;39(4):547–62.
15. Yoo JH, Lee JS, Lee YS, Ku S, Lee HJ. Protective effect of bovine milk against HCl and ethanol–induced gastric ulcer in mice. J Dairy Sci. 2018 May;101(5):3758–70.
16. Linda Costanzo. BRS Physiology Costanzo. 7th ed. China: Elsevier; 2021.
17. Torjesen I. Gastric acid drugs linked to increased risk of Clostridium difficile infection. Pharm J. 2017;
18. Engevik AC, Kaji I, Goldenring JR. The Physiology of the Gastric Parietal Cell. Physiol Rev. 2020 Apr 1;100(2):573–602.
19. Moriguchi T, Takai J. Histamine and histidine decarboxylase: Immunomodulatory functions and regulatory mechanisms. Genes to Cells. 2020 Jul 12;25(7):443–9.
20. Savarino E, Marabotto E, Zentilin P, Furnari M, Bodini G, Pellegatta G, et al. A safety review of proton pump inhibitors to treat acid-related digestive diseases. Expert Opin Drug Saf. 2018 Aug 3;17(8):785–94.
21. Strand DS, Kim D, Peura DA. 25 Years of Proton Pump Inhibitors: A Comprehensive Review. Gut Liver. 2017 Jan 1;11(1):27–37.
22. Freedberg DE, Kim LS, Yang YX. The Risks and Benefits of Long-term Use of Proton Pump Inhibitors: Expert Review and Best Practice Advice From the American

Gastroenterological Association. Gastroenterology. 2017 Mar 1;152(4):706–15.
23. Yu LY, Sun LN, Zhang XH, Li YQ, Yu L, Yuan ZQY, et al. A Review of the Novel Application and Potential Adverse Effects of Proton Pump Inhibitors. Adv Ther. 2017 May 1;34(5):1070–86.
24. Srinutta T, Chewcharat A, Takkavatakarn K, Praditpornsilpa K, Eiam-Ong S, Jaber BL, et al. Proton pump inhibitors and hypomagnesemia. Medicine. 2019 Nov;98(44):e17788.
25. Poly TN, Islam MM, Yang HC, Wu CC, Li YC(. J). Proton pump inhibitors and risk of hip fracture: a meta-analysis of observational studies. Osteoporos Int. 2019 Jan 18;30(1):103–14.
26. Ghebre YT. Proton Pump Inhibitors and Osteoporosis: Is Collagen a Direct Target? Front Endocrinol (Lausanne). 2020 Jul 22;11.
27. Nassar Y, Richter S. Proton-pump Inhibitor Use and Fracture Risk: An Updated Systematic Review and Meta-analysis. J Bone Metab. 2018;25(3):141.
28. Shiraev TP, Bullen A. Proton Pump Inhibitors and Cardiovascular Events: A Systematic Review. Heart Lung Circ. 2018 Apr;27(4):443–50.
29. Azadiradione | C28H34O5 - PubChem [Internet]. [cited 2022 Nov 27]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/12308714#section=Chemical-Target-Interactions
30. Gupta SC, Prasad S, Tyagi AK, Kunnumakkara AB, Aggarwal BB. Neem (Azadirachta indica): An indian traditional panacea with modern molecular basis. Phytomedicine. 2017 Oct;34:14–20.
31. Islas JF, Acosta E, G-Buentello Z, Delgado-Gallegos JL, Moreno-Treviño MG, Escalante B, et al. An overview of Neem (Azadirachta indica) and its potential impact on health. J Funct Foods. 2020 Nov;74:104171.