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PHARMACOLOGICAL SCREENING OF ANTIULCER AGENTS: A REVIEW

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ABSTRACT

Gastric hyperacidity and gastro duodenal ulcer is a very common global problem today. Among various causes of gastric ulceration lesions caused by stress, alcohol consumption, *H.pylori* infection and use of NSAIDs have been shown to be mediated largely through the generation of reactive oxygen species (ROS), especially hydroxyl radical (*OH). Hyper secretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of gastric mucosa through the proton pumping H+ K+ ATPase. The modern approach to control gastric ulcer is to inhibit gastric acid secretion, to promote gastro protection, to block apoptosis and stimulate epithelial cell-proliferation for effective healing. On contrary most of the green pharmaceuticals reduces the offensive factors and have proved to be safe, clinically effective, better patient tolerance, relatively less expensive and globally competitive.

INTRODUCTION: Gastro-intestinal disorders are one of the severe classes of human ailments causing maximum discomfort, morbidity and mobility. Peptic ulcer is one such git disorder. Peptic ulcer is benign lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed acid and pepsin. There are several causes including, stress, alcohol consumption, Cigarette smoking, H.pylori infection, ingestion of drugs and chemicals. Especially consumption of alcohol for a prolonged period, smoking of Cigarette, or chronic consumption of NSAIDs are causing peptic ulcers. The role of free radicals in the pathogenesis of peptic ulcer due to mucosal damage is established. The symptoms of peptic ulcer are: severe pain and irritation in the upper abdomen. If it is not treated properly, it may result in perforations in the wall of the gastrointestinal tract.

PEPTIC ULCER: It is a chronic inflammatory condition involving a group of disorders characterized by ulceration in regions of upper gastrointestinal tract where parietal cells secret pepsin and hydrochloric acid [1].

Signs and symptoms: Here in peptic ulcer diseases patients can be asymptomatic or experience anorexia, nausea, vomiting, bleaching and blotting and heart burn or epigastric pain [1].

Epidemiology: The life time prevalence of peptic ulcer diseases is 5 to 10% in the general population. There are approx 3.9 million patients with peptic ulcer diseases in United States with 200,000 to 400,000 new cases reported each year. The peak incidence is between 50 to 70 years of age [1].

Etiology of chronic ulceration[2]:

Heredity: Patients with peptic ulcer often have a family history of the diseases. This is particularly the case with duodenal ulcers which develop below the age of 20 years. The gastric ulcer patients have 3 times the expected number of gastric ulcer but duodenal ulcer occurs with the same frequency amongst relatives as in the general population.

Acid-pepsin Vs mucosal resistance: The immediate cause of peptic ulceration is digestion of the mucosa by acid and pepsin of the gastric juice. But the sequence of events leading to this is unknown. Digestion by acid and pepsin cannot be the only factor involved, since the normal stomach is obviously capable of resisting digestion by its own secretion. The concept of ulcer etiology may be written as “acid plus pepsin Vs mucosal resistance”.

Gastric hyper secretion: Ulcer occurs only in the presence of acid and pepsin. They are never found in achlorhydric patients such as those with pernicious anaemia. Acid secretion is more important in the aetiology of duodenal than gastric ulcer. Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the side effects like arrhythmias, impotence, gynacomastia and haematopoietic changes of synthetic drugs [2] hence there usage for a chronic period is restricted.

DIFFERENT FACTORS RELATED TO ACID SECRETION (Shown in figure 1)[3]:

General factors: Vagal hormonal effect, histamine and epinephrine, insufficient circulation, shock and general ischemia increase the secretion.

- Constitutional and environmental factors i.e. sex, age, temperature, family history, social class, geographical differences; occupation may also influence the acid release.

- Local factors in stomach.

Aggressive factors: HCl, pepsin, refluxed bile, NSAIDs, alcohol, pancreatic proteolytic enzymes, ingested irritants, bacterial toxins, physiochemical trauma; all of these factors increase the acid secretion.

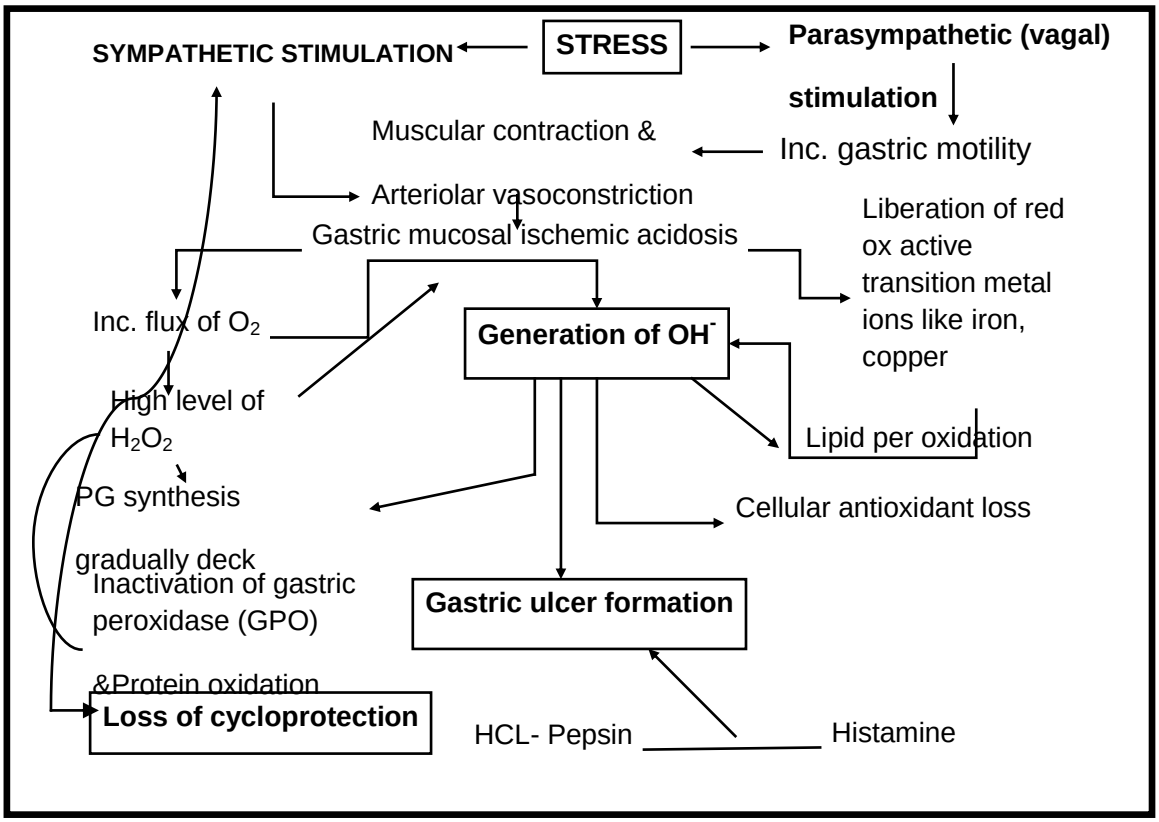

FIGURE 1: PATHOPHYSIOLOGY OF ULCER

Digestive factors: Mucus, bicarbonates, blood flow, resolution of epithelium, the current status of therapy.

ANIMAL MODELS IN EXPERIMENTAL PEPTIC ULCER: Studies on animal models helps to understanding the aetiology and screening of anti-ulcer agents**.**

- Ethanol-induced ulcers
- Cold restraint stress-induced ulcers
- Stress-induced gastric ulceration
- Pylorus ligated(PL)-induced ulcers
- Acetic acid-induced ulcers
- Histamine- induced ulcers
- Indomethacin-induced ulcers
- Serotonin- induced ulcer
- Aspirin-induced ulcers
- Reserpine-inducd ulcers

Alcohol induced gastric ulcer:

Principle[4]: Alcohol causes secretion of gastric juice and decrease mucosal resistance due to which protein content of gastric juice is significantly increased by ethanol. This could be leakage because of plasma protein in the gastric juice with weakening of mucosal resistance

barrier of gastric mucosa, this leading to peptic ulcer.

Procedure[5]: Albino rats of either sex weighing between (150-200 gms) are divided into six groups of animals in each group. The animals are fasted for 24 hours with free access water. Animals are given test drugs or standard drug. 1 hour later 1ml/200gm of 99.80% alcohol is administered p.o to each animal.

Animal are sacrificed 1 hour after alcohol administration, stomach is isolated and cut open along the greater curvature and pinned on a soft board. The length of each gastric lesion is measure in mm. The % inhibition is expressed as sum of the length of the control-mean lesion index of text / mean lesion index of control × 10.

H. pylori- induced gastric ulcer:

Principle[6]: It is a gram negative bacteria found in gastric an duodenal mucosa of most persons particularly the elderly. They, while in the mucosa, split into ammonia and thus elevates the local region of the mucosa by high alkalinity. In this way they strongly help the peptic ulcer development.

Procedure[7]: Albino Wistar rats of either sex weighing between (150-200 gms) are divided into five groups of six animals in group. In this method albino rats are fasted in individual cages for 24 hours. Care was being taken to avoid coprology. Test drug or standard drug or control vehicle is administered 30 minute prior to pyloric ligation. Under light ether anaesthesia, the abdomen is opened and the pylorus was ligated. The abdomen is then sutured. At the end of 4 hours after ligation the animals are sacrificed with excess of anaesthetic ether , and the stomach is dissected out gastric juice is collected were drained into tubes and were centrifuged at 1000 rpm for 10 minutes and the volume is noted. The pH of gastric juice is recorded by pH meter. Then the contents are subjected to analysis for free and total acidity. The stomachs are then washed with running water to see for ulcers in the glandular portion of the stomach.

The numbers of ulcers per stomach are noted and severity of the ulcers scored microscopically with the help of hand lens (10x) and scoring was done as per Kulkarni (1987).

0 = Normal stomach
0.5 = Red coloration
1 = Spot ulcers
1.5 = Haemorrhagic streaks
2 = Ulcer > 3 mm but > 5 mm
3 = ulcers > 5 mm

Percentage protection = 100- ut / uc × 100

Mean ulcer score for each is expressed as ulcer index. The percentage protection is calculated using the above formula.

where, ut = ulcer index of treated group.

uc = ulcer index of control group.

Stress- induced gastric ulcer:

Principle [8]: Stress can arise from prolonged anxiety, tension, and emotion, severe physical discomfort, haemorrhage and surgical shock, burns and trauma, thereby resulting in severe gastric ulceration. The mechanism of gastric ulceration is poorly understood. Recently

research has shown that resistant cold stress causes severe haemorrhage ulcer through derangement of the mucosal antioxidant enzyme such as super oxide, dismutase and peroxides. This is the stress condition arising mainly from physiology discomfort and the mechanism of ulceration caused in this case should be different from ulcer caused due to other factors. The stress generate highly reactive OH* radicals that causes oxidative damage of the gastric mucosa and that the radicals is formed by metal catalysed. Herber weiss reaction between O_2^- and H_2O_2 following induction of the superoxide dimutase and oxidative damage of gastric peroxides.

Procedure[3]: Albino Wistar rats of either sex weighing between (150-200 gms) are divided into five groups of six animals in group.

Cold resistance stress (CRS) induced ulcer-to 18 hours fasted rats, cold resistance stress is given by strapping the rats on a wooden plank and keeping them for 2 hours at 4^o -6^oc. The animals are then sacrificed by cervical dislocation and ulcers are scored on the dissected stomachs.

Aspirin- induced gastric ulcer:

Principle[5]: NSAIDs inhibits the PG synthesis of gastric mucosa, PG gives cytoprotection. Enhancement of leukotriene synthesis, exhibits damage effect. Aspirin also inhibit gastric peroxidase&may increase mucosal H_2O_2 & hydroxyl ions level to cause oxidative mucosal damage.

Procedure[5]: Albino rats of either sex weighing between 150-200 gms are divided into five groups of six animals in group. The animals are fasted for 24 hours. The test drug in varying concentrations based on the design of the experiment is administered orally in 2% gum acacia solution 30 minute prior to aspirin at dose of 200 mg/kg. 4 hours later the rats are sacrificed by using anaesthetic ether and their stomachs dissected. for the determination of gastric lesions.

Parameter studied - ulcer index: The numbers of ulcers per stomach is noted and severity of the ulcers scored microscopically with the help of hand lens (10x) and scoring is done as per Kulkarni (1987).

0 = Normal stomach
0.5 = Red coloration
1 = Spot ulcers
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2 = Ulcer > 3 mm but ‹ 5 mm
3 = ulcers > 5 mm

Percentage protection = 100- ut / uc × 100

Mean ulcer score for each is expressed as ulcer index. The percentage protection is calculated using the formula.

Where, ut = ulcer index of treated group.

Use = ulcer index of control group.

Acetic acid- induced gastric ulcer:

Principle: Acetic acid is reported to produce ulcers by gastric obstruction leading to increase in acidic gastric juice.

Procedure: The rats were anesthetized with phenobarbitone (35 mg/kg, i.p.). the abdomen was opened and the stomach was visualized. Gastric ulcers were produced in rats at the anterior serosal surface of the glandular portion

of the stomach 1 cm away from the pyloric end by 50% acetic acid (0.06ml/animal). Test drug and standard drug was given on day 1,orally, 4h after the application of acetic acid and continued for either up to 3 or 7 days after induction of ulcer. The animals were sacrificed after 18 h of the last dose of test drug either on 4^{th} day or 8^{th} day of experiment to assess the ulcer size and healing.

Indomethacin (IND)-induced gastric ulcers:

Principle[9]: Oral administrations of indomethacin (20 mg/kg) resulted in production of gastric lesions predominantly on glandular segment of the stomach and few or non in the antrum.lesions were produced linearly on mucosal folds and had appearance of mucosal erosions.

Procedure[2]: IND (20mg/kg b.w.) suspended in 0.5% carboxymethyl cellulose was given as a single i.p. dose to induce gastric ulcers after 30 min of test or standard drug treatment. After 5h, the animals were killed and lesions in the gastric mucosa were scored. After identification of ulcer areas, the length of the ulcer was measured along the greater diameter. Number of haemorrhagic spots was considered equivalent to 1mm of ulcer. The mean ulcer size was calculated by dividing the total length (in mm) of ulcers for all the animals divided by total number of animals.

Histamine-induced gastric ulcer:

Principle[10]: Histamine-induced gastric ulceration is recognised to be mediated through both enhance gastric acid secretion and vasosplastic action of histamine.

Procedure: Guinea pigs weighing 300-400 g were fasted for 36 hr. with water ad libitum prior to the experiment and were divided into two groups of six animals each. Gastric ulceration was induced by i.p. administration of histamine acid phosphate (Sigma USA) (50 mg. base.). To protect the animals against histamine toxicity, 5 mg. of promethazine hydrochloride was injected i.p. to each animal 15 min after histamine administration. The test drug or control vehicles (Dist. water) were given orally 45 min before histamine administration[10].

Reserpine-induced gastric ulcer:

Principle: Reserpine-induced gastric ulceration has been attributed to the degranulation of gastric mast cells and consequent liberation of histamine which is believed to be a cholinergacally mediated[10].

Procedure: Adult albino rats were fasted for 24 hr. following water ad libitum. Reserpine (5mg/kg) administered intramuscularly to four groups of six rats each. 30 min after the administration of the test drug or control vehicle (Distilled water) intraperitoneally. All the animals were sacrificed after 18 hr, there stomachs were removed, opened along the greater curvature and sum of lengths(mm) of all lesions for each rat was used as “ulcer index”[10].

Serotonin-induced gastric ulcer:

Principle: Serotonin-induced gastric ulceration is believed to arise from a disturbance of gastric mucosal microcirculation .The development of ulcers by serotonin and reserpine usually takes about 18 hr[10].

Procedure: Serotonin creatinine sulphate (Sigma USA) (20mg/kg) were administered subcutaneously to four groups of rats (24 hr. fasted). The test drug or control vehicle (Distilled water) was administered intraperitoneally after 30 min prior to the serotonin injection. The animals were sacrificed after 18 hr, their stomachs were removed, and the ulcer index was determined as described earlier[10].

PROVED ANTIULCER ACTIVITY OF MEDICINAL PLANTS:

- *Pingamia pinnata*[11].
- *Aloe Vera* [9].
- *Centella asiatica* [12].
- *Eupatorium*[13].
- *Rubia cordifolia*[14] .
- *Piper betle Linn.leaf*[15].
- *Cissampelos mucronata*[16] .
- *Elephatopus scaber Linn*[17] .
- *Haldinia cordifolia*[18].
- *Morinda Citrifolia* [19].
- *Kielmeyera coriacea*[20].
- *Garcinia cambogia*[21].
- *Polyalthia longifolia (Sonn.)*[22].
- *Terminalia chebula*[23].
- *Jasminum grandiflorum L.* [24].
- *Toona ciliata Roemer* [25].
- *Asparagus racemosus Willd* [26].
- *Withania somnifera* [26].
- *Cod Liver Oil* [27].
- *Croton zambesicus* [28].
- *Momordica charantia L* [29].
- *Baccharis dracunculifolia* [30].
- *Bauhinia racemosa* [31].

ESTIMATION OF PARAMETERS:

Estimation of free radical generation: The fundic part of of the stomach is homogenised (5%) in ice cold 0.9% saline with a Potter-Elvehzem glass homogeniser for 30 second. The homogenate is then centrifuged at 800× g for 10 min followed by centrifugation of the supernatant at 12000×g for 15 min and the obtained mitochondrial fraction is used for the following estimation. Statistical analysis was done by student's t-test.

Lipid peroxides (LPO): LPO product malondialdehyde (MDA) is estimated using 1, 1, 3, 3-tetraethoxypropane as the standard and is expressed as n mol/mg protein[2].

Super oxide dismutase (SOD) activity: Inhibition of reduction of nitro blue tetrazolium (NBT) to blue coloured Formosan in presence of phenazine methasulphate (PMS) and NADH is measured at 560 nm using n-butanol as blank. One unit of enzyme activity is defined as the amount of enzyme that inhibits rate of reaction by 50% in one min under the define assay condition and the results have been expressed as unit(U) of SOD activity/mg protein.

Estimation of mucosal glycoprotein's: Sample of gastric mucosal scraping is homogenised in distilled water and treated with 90% ethanol and are subjected for the estimation of carbohydrates and proteins using the methods described above for gastric juice contents. Statistical analysis was done by student's t-test[32].

Estimation of free acidity and total acidity[7]: One ml of gastric juice is pipette into 100 ml conical flask, added 2 to 3 drops of topfer's reagent and

treated with 0.01 N sodium hydroxide until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of the alkali added was noted. This volume corresponds to free acidity. Then 2 to 3 drops of phenolphthalein solution is added and titration is continued until a definite red tinge reappears. Again the total volume of alkali added is noted. Acidity is calculated by using the formula:

Acidity = Volume of NaOH × Normality of NaOH × 100 / 0.1 × meq/L/100 gm.

Estimation of DNA in gastric mucosa: DNA and protein are estimated in the gastric fundal mucosal scrap homogenised in 2.5 ml of ice cooled 0.6N perchloric acid (PCA). The concentration of DNA is expressed as µg DNA/mg protein[32].

Estimation of glandular weights of stomach: The weight of the glandular portion of stomach is calculated by subtracting the weight of the whole stomach minus rumen and is expressed as mg/100 g body weight of the animals. Statistical analysis was done by student's t-test[32].

Estimation of Ulcer index(Ui):

Ui = mean degree of ulceration × % group of ulceration /100
%inhibition = (ulcer index in control - ulcer index in test) ulcer index in control x 100

Estimation of gastrin: In order to determine the gastrin levels in plasma, blood is collected by cardiac puncture, centrifuged, and the plasma is analysed for gastrin levels with double-antibody liquid phase radioimmunoassay[2].

Histological studies:

Gastric tissue samples from each group were fixed in 10% formalin for 24 h. The formalin fixed specimens are embedded in paraffin and section (3-5µm) and stained with haematoxylin and eosin dye. The histochemical sections are evaluated by light microscopy[2].

CONCLUSION: There are several techniques to evaluate the green pharmaceuticals as anti ulcer agents like Aspirin-induced ulcer model, Stress-induced ulcer model, Pylorus lighted-induced ulcer model, Ethanol-induced ulcer model, Acetic acid-induced ulcer model, Cold resistant stress-induced ulcer model, Histamine-induced ulcer model etc. The natural and synthetic products can be scientifically evaluated to establish therapeutic efficacy by the above mentioned techniques.

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