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Review Article

An Overview of *In Vivo* and *In Vitro* Models that can be used for Evaluating Anti-Gastric Ulcer Potential of Medicinal Plants

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Abstract

Many people in the world suffer from peptic ulcers. Of the two main types of peptic ulcers (gastric and duodenal) that develop in humans, gastric ulcers are the most commonly found. Although many medications are currently available for the management of gastric ulcers, prolonged use of these drugs may lead to series of adverse effects such as thrombocytopenia, nephrotoxicity, hepatotoxicity, gynecomastia and impotence. With the increasing tendency for use of herbal drugs for the alleviation of various disease conditions, associated with the belief that natural compounds produce less toxic side effects, much research is now being carried out worldwide, to investigate the potential of plants and plant based medicines to protect against the development of gastric ulcers or alleviate symptoms associated with this condition. In this review, the authors hope to present an overview of the *in vivo* and *in vitro* experimental models that have been used in different laboratories of the world during the past few decades, to carry out such investigations, along with the underlying mechanisms of ulcer induction in each method. The aim is to sensitize other researchers about the different experimental models available for carrying out investigations to discover the gastroprotective potential of plants or herbal remedies used in traditional systems of medicine, for the management of gastric ulcers and develop novel plant based drugs that could be used for their prevention and cure.

Keywords: Gastric ulcer models; Medicinal plants; Gastroprotection; Ulcer index

Introduction

Ulcers are lesions of the skin or mucous membrane characterized by the superficial inflamed dead tissue [1]. Among different types of ulcers that can develop, peptic ulcers are the most common. Peptic ulcers can develop on the inside lining of the stomach (gastric ulcer) or the small intestine (duodenal ulcer) [2]. Gastric ulcer, one of the widest spread, is believed to be due to an imbalance between aggressive and protective factors [3]. Studies have shown that gastric ulcer occurs in at least 10% of the world's population [4]. The major protective factors include adequate blood flow, secretion of prostaglandins, mucin, nitric oxide, bicarbonate and growth factors. Aggressive agents include increased secretion of hydrochloric acid and pepsin, inadequate dietary habits, free oxygen radicals, the consumption of non-steroidal anti-inflammatory drugs and alcohol, stressful conditions and infection with *Helicobacter pylori* [5,6].

Several drugs such as anticholinergic drugs, histamine H2-receptor antagonists, antacids and irreversible proton pump inhibitors have been used for the treatment of gastric and duodenal ulcers [7]. However, prolonged use of these drugs may lead to series of adverse effects such as thrombocytopenia, nephrotoxicity, hepatotoxicity, gynecomastia and impotence [7,8]. Due to such unpleasant side effects produced by conventional drugs, there is an urgent need of more effective and safer treatments with fewer side effects, for the treatment of gastro-duodenal ulcers. Therefore, during the past few years, there has been an increasing interest in the development of plant based gastroprotective agents that are believed to produce less toxic side effects [9]. In this context it is important to first screen plants or herbal remedies with traditional ethno-medicinal uses in gastric ulcer management, for validation of their reputed antiulcer activity. To carry out such investigations it is essential to have credible experimental models.

Several *in vivo* and *in vitro* models are available to evaluate the antiulcer activity of medicines/plants. However, selection of a suitable model has proven to be difficult as each model has significant advantages as well as disadvantages. Further information about these various *in vivo* and *in vitro* models are scattered in the literature, and difficult to find. The main aim of this review is to present to the various researchers interested in carrying out studies on the gastroprotective potential of plants or herbal remedies, a comprehensive overview of available *in vivo* and *in vitro* models that could be used for this purpose, along with the underlying mechanisms of ulcer induction in each method. Thus, it gives a broad view of the issue that will help to select the most appropriate model for the validation of existing traditional therapies for gastric ulcers and development of novel plant based drugs that could be used for their prevention and cure.

In vivo models that can be used for study of gastroprotection

Peptic ulcers can be induced by physiological, pharmacological

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Table 1: Examples of medicinal plants whose gastroprotective activity has been evaluated by use of the absolute ethanol induced gastric lesion model.

Plants	Ref: No.	Type of extract and doses	Type of animal species used in the experiment	Dose of ethanol used to induce ulceration	Reference drug
Acacia ferruginea DC.	[5]	Bark Ethyl acetate fraction of acetone extract (100 mg/kg)	Male Wistar rats (180-220 g)	99.5% absolute ethanol (5mL/kg, p.o)	Omeprazole (20 mg/kg, p.o)
Alpinia calcarata Roscoe	[18]	Rhizomes Hot water extract 500, 750, 1000 mg/kg)	Cross-bred male albino rats (200-250 g)	Absolute ethanol (1 mL/kg, p.o)	Cimetidine (100 mg/kg, p.o)
Cenostigma macrophyllum Tul.	[19]	Leaves Hydroalcoholic fraction of ethanol extract (50, 100, 200 mg/kg)	Male Swiss mice (20-30 g)	Absolute ethanol (0.5 mL, p.o)	Carbenoxolone (100 mg/kg, p.o)
Conyza blinii H. Lev.	[20]	Whole plant Total saponins (5, 10, 20 mg/kg)	Male & Female Sprague - Dawley rats (180-200 g)	Absolute ethanol (1 mL, p.o)	Colloidal bismuth subcitrate (14.4 mg/kg, p.o)
Curcuma xanthorrhiza Roxb.	[21]	Leaves Cold ethanolic extract (250, 500 mg/kg)	Male Sprague - Dawley rats (200-250 g)	Absolute ethanol (5mL/kg, p.o)	Omeprazole (20 mg/kg, p.o)
Margaritaria discoidea Baill.	[22]	Stem bark Ethanolic extract (50, 100, 150 mg/kg) Hexane, ethyl acetate, dichloromethane, butanol and aqueous fractions (150 mg/kg from each fraction)	Male Wistar albino rats (100-150 g)	Absolute ethanol (1mL, p.o)	Misoprostol (0.1 mg/kg, p.o)
Piper tuberculatum Jacq.	[23]	Fruits Dichloromethane fraction of ethanol extract (10, 30, 100 mg/kg)	Female Wistar rats (180 - 220 g)	Absolute ethanol (0.5mL/200g, b.wt, p.o)	Carbenoxolone (100 mg/kg, p.o)
Polygonum chinense Linn.	[24]	Leaves Hot water extract (62.5, 125, 250, 500 mg/kg)	Male & Female Sprague - Dawley rats (200-250 g)	Absolute ethanol (5mL/kg, p.o)	Omeprazole (20 mg/kg, p.o)
Trichosanthes cucumerina Linn.	[25]	Aerial parts Hot water extract (375, 500, 750,1000 mg/kg)	Male & Female Wistar rats (200-225 g)	Absolute ethanol (5mL/kg, p.o)	Cimetidine (100 mg/kg, p.o) Sucralfate (400 mg/kg, p.o)
Vochysia tucanorum Mart.	[26]	Leaves Methanol extract (250, 500, 1000 mg/kg) Butanol fraction of methanolic extract (37.5, 75, 150 mg/kg)	Male Wistar rats (150-250 g)	99.5% absolute ethanol (1 mL, p.o)	Carbenoxolone (100 mg/kg, p.o)
Zanthoxylum rhoifolium Lam.	[27]	Stem bark Ethanol extract (62.5, 125, 250, 500 mg/kg)	Male & female Swiss albino mice (20-30 g)	Absolute ethanol (1mL, p.o)	N- acetylcysteine (750 mg/kg, i.p)

or surgical manipulation in several animal systems. However, rodents are the most commonly used as *in vivo* experimental models. The principles of those that are most frequently used by researchers investigating the gastroprotective effects of plants or herbal remedies, along with their underlying mechanisms of action, are described below.

Absolute ethanol-induced gastric lesions: Gastric mucosal injury may occur when defense mechanisms are impaired by noxious substances such as gastric acid and HCl secretion into the gastric lumen [10]. Administration of absolute ethanol by gavage has long been used as a reproducible method to induce gastric injury in experimental animals [11]. Ethanol can promote the development of gastric lesions by exposing the mucosa to the hydrolytic and proteolytic actions of hydrochloric acid and pepsin [12]. Moreover, ethanol can stimulate gastric acid secretion, resulting in microvascular injuries that facilitate vascular permeability, through reflex release of gastrin and histamine from sensitive nerve terminals present in the gastric mucosa [13]. It is known that intra-gastric administration of ethanol results in gastric mucosal injury characterized by disturbances in microcirculation, mast cell secretary products, inhibition of prostaglandin synthesis, reduction in mucus production and reactive species [14]. Ethanol is also known to increase cellular oxidative stress [15] and produce alterations in gastric cell calcium levels [16] that may lead to the pathogenesis of gastric mucosal injury.

The variety of damaging effects mediated by ethanol has been exploited in developing the ethanol induced gastric ulcer model for testing the gastroprotective potential of various plants/ natural compounds. However, because this model is independent of gastric acid secretion, it is not suitable to evaluate protection against ulceration dependent on acid secretion. Because ethanol can directly enhance the levels of free radicals that can mediate alterations in cell structure and function or contribute to other mechanisms that support oxidative damage [14] and can also mediate direct toxic effects on the gastric mucosa resulting in reduced secretion of bicarbonates and gastric mucous production [17], it is more appropriate to use this model for evaluating the gastroprotective potential of test materials that have cytoprotective and/or antioxidant activities (Table 1).

To induce ulcers with ethanol, rats that have been fasted for 24-36 h are pretreated with vehicle or extracts or reference drug orally. After 1h, ulcers are induced by administration of absolute ethanol orally and kept for another hour after which rats are sacrificed, stomachs excised and severity of ulceration measured.

HCl/ethanol induced gastric lesions: This model may be considered to be an advanced model of the absolute ethanol induced ulcer model discussed before. Instead of ethanol only, a mixture of HCl and ethanol are used to induce ulceration. Gastric lesions induced by HCl/ethanol are due to direct necrotizing action on the gastric mucosa. Combination of ethanol with HCl is considered to

Table 2: Examples of medicinal plants whose gastroprotective activity has been evaluated by use of the HCl/ethanol.

Plants	Ref: No.	Type of extract and doses	Type of animal species used in the experiment	Dose of HCl/ethanol used to induce ulceration	Reference drug
Celtis iguaneae Jacq.	[29]	Leaves Hexane extract (100 mg/kg)	Male albino Swiss mice (25-35 g)	0.45M HCl/60 % ethanol solution (10 mL/kg, p.o)	Carbenoxolone (200 mg/kg, p.o)
Cenostigma macrophyllum Tul.	[19]	Leaves Hydroalcoholic fraction of ethanol extract (50, 100, 200 mg/kg)	Male Swiss mice (20-30 g)	0.15M HCl/40% ethanol solution (1 mL, p.o)	Carbenoxolone (100 mg/kg, p.o)
Centaurea solstitialis Linn.	[30]	Flowers Sesquiterpene lactones: chlorojanerin (59.2 mg/kg) 13- acetyl solstitialin (179 mg/kg)	Male & Female Balb-C mice (20-40 g)	0.3M HCl/50 % ethanol solution (0.2 mL, p.o)	Famotidine (20 mg/kg, p.o)
Copaifera langsdorffii Desf.	[6]	Leaves 70% aqueous ethanolic extract(50,250,500 mg/kg) Isolated compounds: α humulene, β caryophyllene oxide, kaurenoic acid, quercitrin and afzelin (30 mg/kg/compound)	Swiss mice (25-30 g)	0.3M HCl/50% ethanol solution (0.2 mL, p.o)	Omeprazole (30 mg/kg, p.o)
Helicteres sacarolha A. St.-Hil.	[31]	Leaves Hydroethanolic extract (20, 50, 250 mg/kg)	Albino mice-Swiss-Webster strain (25-30 g)	0.3M HCl/60% ethanol solution (0.3 mL, p.o)	Carbenoxolone (100 mg/kg, p.o)
Vochysia tucanorum Mart.	[26]	Leaves Methanol extract (250, 500, 1000 mg/kg)	Male Swiss mice (25-40 g)	0.3M HCl/60% ethanol solution (0.2 mL, p.o)	Lansoprazole (30 mg/kg, p.o)
Zanthoxylum rhoifolium Lam.	[27]	Stem bark Ethanol extract (62.5, 125, 250, 500 mg/kg)	Male & female Swiss albino mice (20-30 g)	0.15M HCl/40% ethanol solution (1 mL, p.o)	N- acetylcysteine (750 mg/kg, i.p)

Table 3: Examples of medicinal plants whose gastroprotective activity has been evaluated by use of the stress models.

Plants	Ref: No.	Type of extract and doses	Type of animal species used in the experiment	Temperature	Reference drug
Water-immersion stress model					
Centaurea solstitialis Linn.	[30]	Flowers Sesquiterpene lactones: chlorojanerin (59.2 mg/kg) 13- acetyl solstitialin (179 mg/kg)	Male & Female Sprague-Dawley arts (120-200 g)	19-22 °C	Famotidine (20 mg/kg, p.o)
Copaifera malmei Harms.	[38]	Leaves Infusion (25, 100, 400 mg/kg)	Male & Female Wistar albino arts (120-200 g)	19 ± 1 °C	Cimetidine (100 mg/kg, p.o)
Helicteres sacarolha A. St.-Hil.	[31]	Leaves Hydroethanolic extract (20, 50, 250 mg/kg)	Wistar strain rats (180-220 g)	22 °C	Ranitidine (50 mg/kg, p.o)
Sambucus ebulus Linn.	[39]	Leaves n-buatnol fraction (540, 816 mg/kg) and remaining water sub extract (843 mg/kg) of methanolic extract	Male Wistar strain rats (120-150 g)	17-22 °C	Famotidine (2 mg/kg, p.o)
Cold-resistant stress model					
Celtis iguaneae Jacq.	[29]	Leaves Hexane extract (100 mg/kg)	Male albino Swiss mice (25-35 g)	4 °C for 2 h	Ranitidine (100 mg/kg, p.o)
Cenostigma macrophyllum Tul.	[19]	Leaves Hydroalcoholic fraction of ethanol extract (50, 100, 200 mg/kg)	Male Swiss mice (20-30 g)	4 ± 1 °C for 3 h	Cimetidine (100 mg/kg, p.o)
Zanthoxylum rhoifolium Lam.	[27]	Stem bark Ethanol extract (125, 250, 500 mg/kg)	Male & female Wistar rats (180-240 g)	3 ± 1 °C for 4 h	Cimetidine (100 mg/kg, p.o)

accelerate the progress of ulcerogenesis and enhance gastric injury [28].

In this model, 18-24 h fasted mice are pretreated with vehicle or extracts or reference drug orally. After 1 h, gastric lesions are induced by administrating the ethanol/HCl mixture. The animals are euthanized after 30 min or 1 h, stomachs excised and severity of ulceration measured (Table 2).

Water-immersion stress or cold-resistant stress induced gastric ulcers: Gastric ulcers induced by water –immersion stress or cold resistant stress [32] in rats or mice are known to resemble human peptic ulcers, both grossly and histopathologically [33]. The restraint technique developed by Brodie and Hanson [34] when coupled with the cold water or ordinary water immersion method developed by Levine [35] is reported to induce stress lesions in a synergistic manner.

Stress induced ulcers are mediated mainly by the release of histamine that results in an increased acid secretion, decreased mucus production, pancreatic juice reflux and poor flow of gastric blood. Generation of reactive oxygen species and inhibition of prostaglandin synthesis also promote stress induced ulcer formation [36,37]. This model has been widely used for assessing the gastroprotective effects of various test agents (Table 3), especially those with mucus enhancing and cytoprotective properties.

In water-immersion stress model, animals are fasted for a period of 24-36 h prior to the experiment and treated with vehicle or test

Table 4: Examples of medicinal plants whose gastroprotective activity has been evaluated by use of the pylorus-ligated-induced peptic ulcer model.

Plants	Ref: No.	Type of extract and doses	Type of animal species used in the experiment	Reference drug
Margaritaria discoidea Baill.	[22]	Stem bark Ethanolic extract (50, 100, 150 mg/kg)	Male Wistar albino rats (100-150 g)	Cimetidine (100 mg/kg, p.o)
Muntingia calabura Linn.	[41]	Leaves Methanolic extract (100, 200, 500 mg/kg)	Male Sprague Dawley rats (180-200 g)	Cimetidine (100 mg/kg, p.o)
Piper tuberculatum Jacq.	[23]	Fruits Dichloromethane fraction of ethanol extract (10, 30, 100 mg/kg)	Female Wistar rats (180-220 g)	Piplartine (15 mg/kg, p.o)
Sambucus ebulus Linn.	[39]	Leaves n-buatnol fraction (540, 816 mg/kg) and remaining water sub extract (843 mg/kg) of methanolic extract	Male Wistar strain rats (120-150 g)	Famotidine (2 mg/kg, p.o)
Trichosanthes cucumerina Linn.	[42]	Fruits 50% ethanol extract (500 mg/kg)	Male & female Wistar rats (200-250 g)	Cimetidine (100 mg/kg, p.o)
Zanthoxylum rhoifolium Lam	[27]	Stem bark Ethanol extract (125, 250, 500 mg/kg)	Male & female Wistar rats (180-240 g)	Cimetidine (100 mg/kg, p.o)

Table 5: Examples of medicinal plants whose gastroprotective activity has been evaluated by use of NSAID's.

Plants	Ref: No.	Type of extract and doses	Type of animal species used in the experiment	Dose of NSAID to induce ulceration	Reference drug
Cenostigma macrophyllum Tul.	[19]	Leaves Hydroalcoholic fraction of ethanol extract (100 mg/kg)	Wistar rats (200-250 g)	Indomethacine (30 mg/kg, s.c.)	Cimetidine (100 mg/kg, p.o)
Copaifera malmei Harms.	[38]	Leaves Infusion extract (25,100, 400 mg/kg)	Male & Female Wistar rats (180-220 g)	Piroxicam (200 mg/kg, p.o)	Ranitidine (100 mg/kg, p.o)
Trichosanthes cucumerina Linn.	[25]	Aerial parts Hot water extract (750 mg/kg)	Male & Female Wistar rats (200 -225 g)	Indomethacine (25mg/kg, p.o)	Cimetidine (100 mg/kg, p.o)
	[42]	Fruits 50% ethanolic extract (500 mg/kg)	Wistar rats (200-250 g)	Aspirin (500 mg/kg, p.o)	Cimetidine (100 mg/kg, p.o)
Margaritaria discoidea Baill.	[22]	Stem bark Ethanolic extract (50, 100, 150 mg/kg)	Male Wistar albino rats (100-150 g)	Indomethacine (80 mg/kg, p.o)	Omeprazole (100 mg/kg, p.o)
Glycyrrhiza glabra Linn.	[48]	Roots & Rhizomes 70 % aqueous ethanolic extract (50,100,150 & 200 mg/kg)	Male & Female Swiss mice (25-30 g)	Indomethacine (60 mg/kg, p.o)	Cimetidine (100 mg/kg, p.o)

drug or reference drug. After 30 min. animals are placed individually in each compartment of a stress cage and immersed vertically up to the xyphoid level in a water bath and kept for 7 h to induce stress ulcer. After 7 h animals are sacrificed and ulceration quantified.

In cold resistant stress model animals are fasted for a period of 18 h prior to the experiment treated with vehicle or test drug or reference drug. After 1 h, rats are individually restrained in plastic cages in a refrigerator for 2-4 h and sacrificed. Finally, stomachs are taken out and severity of ulceration measured.

Pylorus-ligated-induced peptic ulcer: The ligation of the pylorus end of the stomach causes accumulation of gastric acid in the stomach which in turn produces ulcers. Ulcers result from a breakdown of the gastric mucosal barrier resulting from auto digestion of the gastric mucosa. This model is useful for evaluating the (a) cytoprotective effects of drugs that increase secretion of mucus and (b) anti-secretory drugs that reduce secretion of gastric aggressive factors such as acid and pepsin.

Pylorus ligation is performed according to the method described by Shay et al. [40] with slight modifications. In brief, animals fasted for 48 h, are pretreated with vehicle or extracts or reference drug orally, and after 1 h pyloric end of the stomach is ligated under ether anesthesia (Table 4). Stomach is then placed carefully in the abdomen and the wound sutured by interrupted sutures. Animals are sacrificed after 4 h and their stomachs are removed and longitudinally excised along the greater curvature. The inner surface is then examined for ulcerative lesions. The gastric volume and acidity are then assessed according to method described by Shay et al. [40].

NSAID's induced gastric ulcers: Gastric ulcers are known to be induced by excessive use of many Non-Steroidal Anti-Inflammatory Drugs (NSAID's) such as indomethacin, aspirin and ibuprofen [43]. This property has been exploited for the development of NSAID's induced gatric ulcer models in rats. This is one of the most commonly used models for investigating antiulcer properties of test agents. NSAID's induce gastric ulcers by inhibiting prostaglandin synthesis via the cyclooxygenase pathway [43,44]. In the stomach, prostaglandins protect against mucosal injury by stimulating bicarbonate and mucus secretion, maintaining mucosal blood flow and regulating mucosal cell turnover and repair [45,46]. NSAID's can also induce mucosal damage by enhancement of reactive oxygen free radical production and neutrophil infiltration [46]. Furthermore, NSAID's (especially those of acidic nature) can exert direct cytotoxic effects on epithelial cells and disrupt surface active phospholipids on the mucosal surface, independent of effects on synthesis of prostaglandins, thus making the mucosa more susceptible to damage by luminal acid [47]. This model is therefore useful for evaluating the ability of antisecretory

Table 6: Examples of medicinal plants whose gastroprotective activity has been evaluated by use of serotonin.

Plants	Ref: No.	Type of extract and doses	Type of animal species used in the experiment	Dose of serotonin to induce ulceration	Reference drug
Centaurea solstitialis Linn.	[30]	Flowers Sesquiterpene lactones: chlorojanerin (59.2 mg/kg) 13-acetyl solstitialin (179 mg/kg)	Male & Female Balb-C mice (20-40 g)	50 mg/kg (0.5 mL, s.c)	Famotidine (20 mg/kg, p.o)
Ocimum sanctum Linn.	[52]	Seeds Fixed oil (1, 2 mL/kg)	Wistar strain albino rats (160-200 g)	20 mg/kg (0.5 mL, s.c)	Not stated
Sambucus ebulus Linn.	[39]	Leaves n-buatnol fraction (495 mg/kg)	Male Wistar strain rats (120-150 g)	50 mg/kg (0.5 mL, s.c)	Famotidine (2 mg/kg, p.o)

Table 7: Examples of medicinal plants whose gastroprotective activity has been evaluated by use of the acetic acid model.

Plants	Ref: No.	Type of extract and doses	Type of animal species used in the experiment	Dose of acetic acid to induce ulceration	Reference drug
Celtis iguaneae Jacq.	[29]	Leaves Hexane extract (100 mg/kg)	Male albino Swiss mice (25-35 g)	50 µL of 20% acetic acid (sub mucosal injection)	Ranitidine (50 mg/kg, p.o)
Copaifera malmei Harms.	[38]	Leaves Infusion (12.5, 50, 200 mg/kg)	Male & Female Swiss albino mice (25-30 g)	50 µL of 30% acetic acid (sub mucosal injection)	Cimetidine (50 mg/kg, p.o)
Ocimum sanctum Linn.	[53]	Leaves Cold ethanolic extract (50, 100 mg/kg)	Male & Female Sprague Dawley rats (180-200 g)	60 µL of 40% acetic acid (local application to serosal surface of the stomach)	Omeprazole (10 mg/kg, p.o)

Table 8: Examples of medicinal plants whose gastroprotective activity has been evaluated by use of the diethyldithiocarbamate model.

Plants	Ref: No.	Type of extract and doses	Type of animal species used in the experiment	Dose of diethyldithiocarbamate to induce ulceration	Reference drug
Centaurea solstitialis Linn.	[30]	Flowers Sesquiterpene lactones: chlorojanerin (59.2 mg/kg) 13- acetyl solstitialin (179 mg/kg)	Male & Female Balb-C mice (20-40 g)	800 mg/kg (1 mL, s.c)	Not stated
Momordica charantia Linn.	[60]	Fruits Ethanol extract (310, 620 mg/kg)	Male & Female Wistar rats (120-150 g)	800 mg/kg (1.5 mL, s.c)	Not stated

and cytoprotective agents to protect against gastric ulcer development (Table 5).

To evaluate gatroprotective potential of test agents, rats fasted for 24-36 h are orally administered the selected NSAID dissolved in an appropriate vehicle (e.g. water, 1% carboxymethyl cellulose) and after 1 h treated with the different doses of the test agent. After 4 h post treatments with the test agent, rats are sacrificed, stomachs excised and severity of ulceration measured.

Histamine-induced gastric ulcers: Histamine is known to be one of the important factors mediating formation of gastric ulcers, and this is the basis of the histamine induced gastric ulcer model [49]. Histamine released from mast cells binds with receptors present on the surface of parietal cells and causes activation of adenylate cyclase which converts ATP into c-AMP. This conversion is responsible for enhanced secretion of HCl from parietal cells [50]. Histamine also has vasodilating ability and reduces mucus production. These pharmacological effects of histamine have been exploited in producing the histamine-induced ulcer model. This model is useful for evaluating the antisecretory effects of drugs and other agents that function as H2-receptor antagonists.

To induce ulcers with histamine, animals fasted for 18- 24 h are subcutaneously administered histamine phosphate. The animals are sacrificed after 2 h or 4 h following histamine administration and the stomachs dissected out after pylorus and cardiac ligation [51]. The gastric content is collected into a centrifuge tube for determination of pH and total acidity.

This model has been used to evaluate gastroprotection by fixed oil (3 mL/kg) [52] and cold ethanolic leaf extract of *Ocimum sanctum* Linn. (50, 100 mg/kg) [53]. Gastric ulceration was induced by intraperitoneal administration of histamine acid phosphate using male and female guinea pigs (300-350 g). Omeprazole (10 mg/kg p.o) was used as reference drug [53].

Serotonin-induced gastric ulcers: Serotonin is a vasoconstrictor and ulceration induced by serotonin is believed to arise from a disturbance of gastric mucosal microcirculation [52]. In this model, rats fasted for 24 h are pretreated with vehicle or extracts or reference drug. After 30 min serotonin creatinine sulphate (20-50 mg/kg) is administered subcutaneously and animals sacrificed (Table 6). Their stomachs longitudinally excised after 6-18 h along the greater curvature. The inner surface is then examined for ulcerative lesions [54,55].

Acetic acid-induced gastric ulcers: Takagi et al. [56] developed a model for inducing chronic gastric ulcers in rats by sub-mucosal injection of acetic acid. These ulcers resemble chronic ulcers in humans both grossly and histologically. To overcome certain problems, the original method has undergone several modifications and the one that is currently most favoured is that developed by Okabe and Pfeiffer [57]. This method involves application of acetic acid solution intraluminally. The acetic acid induced ulcer model is most suitable for evaluating the effects of various test agents on the

healing process of chronic peptic ulcers and also for screening their antisecretory and cytoprotective effects (Table 7).

Diethyldithiocarbamate induced gastric ulcers: The diethyldithiocarbamate model is useful to assess whether antioxidant activity is a mechanism by which a test agent mediates gastroprotection [58] and to evaluate the cytoprotective potential of test agents. Antral lesions are induced by diethyldithiocarbamate through the mobilization of superoxide and hydroxyl radicals [59].

In this model, animals are fasted for 24 h and water with held for 2 h before commencement of experiment. Acute glandular lesions are then induced by subcutaneous injection of 1 mL of diethyldithiocarbamate in saline followed by oral dose of 1 mL of 0.1N HCl (Table 8).

In vitro methods for evaluation of gastroprotection

Acidity is a common gastrointestinal problem which is attributed to a functional disorder that can result due to a variety of reasons [61]. Antacids act by neutralizing gastric acid and thereby reduce the gastric pH. For preliminary screening of plant extracts to determine their ability to reduce gastric acidity, the following *in vitro* methods have been developed:

1. Neutralizing effects on artificial gastric acid
2. Detection of the duration of consistent neutralization on artificial gastric acid using the modified model of Vatier's artificial stomach
3. Neutralizing capacity *in vitro* using a titration method of Fordtran's model
4. Assessment of H^+ K^+ -ATP'ase activity

Neutralizing effects on artificial gastric acid: The freshly prepared plant extracts (in 90 mL) or distilled water (90 mL) or reference drug (90 mL) is added separately to the artificial gastric juice (100 mL) at pH 1.2. The pH values are determined to examine the neutralizing effects on artificial gastric juice.

For preparation of artificial gastric juice, two grams of NaCl and 3.2 mg of pepsin are dissolved in 500 mL distilled water. Hydrochloric acid (7.0 mL) and adequate water are added to make a 1000 mL solution. The pH of the solution is adjusted to 1.2 [62].

Above model is further developed for the assessment of the duration of consistent neutralization on (a) artificial gastric acid using the modified model of Vatier's artificial stomach and (b) neutralization capacity using the titration method of Fordtran's model described below.

Detection of the duration of consistent neutralization on artificial gastric acid using the modified model of Vatier's artificial stomach: The apparatus of the modified model of Vatier's artificial stomach [63] is made up of 3 elements: a pH recording system (R), a stomach (S) and a peristaltic pump (P). The stomach is made up of 3 portions, S_1, S_2 and S_3 modeled as reservoir, secretary flux and gastric emptying flux respectively.

Each freshly prepared plant extracts (in 90 mL) or distilled water (90 mL) or reference drug (90 mL) is added to 100 ml of artificial gastric juice at pH 1.2 in the reservoir of the artificial stomach at 37 °C and continuously stirred (30 rpm) with 2.5 cm magnetic stirring apparatus. Artificial gastric juice at pH 1.2 is pumped at 3 mL/min into the reservoir of the artificial stomach and pumped out at 3 mL/min at the same time.

A pH meter is connected to continuously monitor the changes of pH in the reservoir of the artificial stomach. The duration of the neutralization effect is determined when the pH value is returned to its initial value (pH 1.2).

Neutralizing capacity *in vitro* using a titration method of Fordtran's model: Each freshly prepared plant extracts (in 90 mL) or distilled water (90 mL) or reference drug (90 mL) is placed in a 250 mL beaker and warmed to 37 °C. A magnetic stirrer is continuously run at 30 rpm to imitate the stomach movements [64].

Each freshly prepared plant extracts or distilled water or reference drug is titrated with artificial gastric juice to the end point of pH 3. The consumed volume (v) of the artificial gastric juice is measured and total consumed H^+ (mmoL) is calculated as 0.063096 (mmoL/mL) × v (mL).

As reference drugs, sodium bicarbonate [62,65] and combination of aluminum hydroxide and magnesium hydroxide [65] can be used.

Examples of plants that have been evaluated using the above models include *Garcinia indica* [62], *Tephrosia calophylla* Bedd. and *Tephrosia maxima* Linn. [65], *Tephrosia* purpurea Linn. [66], and *Laportea aestuans* Linn. [67].

Assessment of H^+ K^+-ATP'ase activity: Gastric mucosal injury may occur when defense mechanisms are impaired by noxious substances, such as gastric acid, HCl secretion into the gastric lumen. The acid secretion is achieved by the enzyme H^+, K^+ - ATP'ase, which catalyzes the exchange of one H^+ for one K^+ at the expense of an ATP molecule [10].

This principle has been utilized by Kubo et al. [68] to develop an *in vitro* method that can be used for evaluating the ability of test materials to reduce gastric acid secretion.

Examples of plants evaluated using this method include *Piper tuberculatum* Jacq. [23], *Cleome viscose* Linn. [69] and *Trametes lactinea* Berk [70].

Assessment of anti- *Helicobacter pylori* activity

The Gram negative bacterium *Helicobacter pylori* that colonize the stomach of many humans are considered to play an important role in the pathogenesis of gastritis and peptic ulcer disease [71]. This organism adheres to mucus that covers gastric epithelial cells and obtains nutrition resulting in damage to the stomach lining. Pathogenesis is mediated by the bacteria ureases. Therefore, many investigators trying to discover natural gastroprotective agents are also currently making efforts to discover potential anti-*H. pylori* agents from medicinal plants and traditional medicines.

In vitro, *H. pylori* is sensitive to a wide range of antimicrobials and this can be readily assessed using standard techniques such as determining MIC and minimum bacteriocidal concentration (MBCs) [72,73]. New methods such as flow cytometry are also being used for rapid high through put screening of antibacterial activity because large numbers of compounds can be assessed and compared by these methods [72].

To evaluate anti-*H. pylori* activity, the broth dilution method as described by Palacios-Espinosa and co-workers [73] can be used. Examples of plants shown to exert anti-*H. pylori* activity include *Impatiens balsamina* Linn., *Foeniculum vulgare* Mill, *Annona cherimola* Mill, *Myristica fragrans* Houtt, *Curcuma amada* Roxburgh, *Magnolia officinalis* Rehder & Wilson, *Schisandra chinensis* Turcz., and *Elettaria cardamomum* Linn. [74].

Conclusion

To validate the presence of gastroprotective properties of medicinal plants used by traditional medical practitioners to treat gastric ulcers, or to discover leads for the development of novel agents from plants for gastric ulcer therapy, it is essential to carry out scientifically controlled investigations to determine if these plant materials truly have the ability to protect against the development of gastric ulcers or alleviate the signs and symptoms associated with this condition. A variety of *in vivo* and *in vitro* models have been developed over the years in different laboratories of the world, for this purpose. However, selection of a suitable model has proven to be difficult, as each model has significant advantages as well as disadvantages. The *in vitro* methods that have been developed may be more useful for preliminary screening of test materials for the presence of antisecretory, antioxidant, or cytoprotective properties that need to be present in an agent that can help protect against gastric ulcer development. However, such *in vitro* experiments should ideally be followed by *in vivo* testing to truly understand how the test agent will behave within the complex environment of the whole body. *In vivo* model may also be used to assess any toxic side effects that may be produced by the test agent.

This review has attempted to present to the readers an overview of the *in vivo* and *in vitro* models that are most frequently being used by investigators for studies concerned with gastroprotection. By use of such methodologies, a number of medicinal plants have been demonstrated to possess significant gastroprotective properties and rationalizes their ethnopharmacological uses. Extracts of plants demonstrating such properties should be further developed into forms that can be conveniently used in clinical practice for the management of gastric ulcers or as adjuncts to existing therapies to reduce their toxic side effects.

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