Review Article

Pharmacological Screening Techniques for Evaluation of Gastric Ulcers: Principles, Mechanism and Procedures

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ABSTRACT

A gastric ulcer is the most common disease in the world. Different allopathic medicine or drugs are used for the treatment of gastric ulcers. These are effective in the treatment of ulcers but produce various side effects. These days use alternative medicine for the treatment of gastric ulcers. The natural drugs produce lower side effects in the living system therefore these are best from allopathic drugs. Identification of plants for the treatment of gastric ulcers the screenings of plants are requiring. Various methods are used to check the activity of natural drugs or herbs. These methods are called experimental animal's models. In pursuing medical knowledge and alleviating human suffering, animal models have provided invaluable details. Using different animal models, the basis of our basic knowledge of disease pathophysiology and human anatomy can primarily be traced to preclinical studies.

Keywords: Pharmacological Screening; Models; Gastric ulcers; Principles; Mechanism.

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INTRODUCTION

A gastric ulcer is the mucosal lesions in which damage the mucosal layer and form a cavity covered by chronic and acute inflammation. Gastric ulcers may locate at the stomach or duodenum. Stomach ulcers are called peptic ulcers and intestine ulcers called duodenal ulcers. Duodenal ulcers are located at the duodenal bulb.[1] A peptic ulcer is the most predominant gastric disease.[2] 10 % of the world population suffered from gastric ulcer disorder.[3] H. pylori induce the damage gastric mucosa layer and increase skin lesions. Regular intake of NSAIDS cause inhibits prostaglandin secretion and result development of gastric ulcers.[4] Peptic ulcers are caused by a disturbance of the balance between gastric protective factors and gastric aggressive factors. If the secretion of the pepsin and hydrochloric acid from parietal cells increases then increase the chances of development of gastric ulcers.[5] Peptic ulcer disease may be identified based on the presence of clinical symptoms such as acidity, dyspepsia, nocturnal pain, reduced pain after food and epigastric burning pain. Some other symptoms such as vomiting, weight loss, anorexia and gastric bleeding observe in some patients. Enzyme-linked immunosorbent assay is used for the identification of *H. pylori* infection.[6]

Different types of antiulcer drugs can use for the management of gastric ulcers. If patients suffer from peptic ulcer disease then not administers alcohol, Smoking and NSAIDs. Drugs such as antihistamine, Proton pump inhibitors, Antacids, anti-microbial and anticholinergic commonly use for the management of peptic ulcers.[7] Proton pump inhibitors are more effective than H_2 receptor antagonists.[8] Plants are the natural source of the drugs that show different pharmacological activities and traditional uses. Some plants are traditionally used for the treatment of gastric ulcers or duodenal ulcers with minimum side effects.[9] For the identification of the property of the meditational plants, screening is required. Different animal experimental models are used for the screening of the plant activities.[10]

Several antiulcer animal models were used for the screening of antiulcer activity of the Phytochemicals of the plants. The choice of the experimental model depends on the experimental design and objective of the studies.

EXPERIMENTAL ANIMAL MODELS

Gastric ulcers can be produced by surgical procedure, physiological methods and pharmacological or drugs in rats, mice, and other several species. There are following screening methods that are used for evaluation of the antiulcer activity of the drugs.

Acetic acid-H. pylori-induced ulcers

Acetic acid and H. pylori use for the induction of gastric ulcers in the experimental animals. This process was performed under anesthesia and laparotomy in rats.[11]

Procedure

Firstly, the male experimental rats (200-250 g) select for this experiment. The animals divide into groups. Group first (normal control) administer the normal saline solution, group second with H. pylori (ulcer control)

administer test drug and group third administer the standard drug (clarithromycin 25 mg/kg).

The rats anesthetized intramuscularly by a mixture of zoletil (12.5 mg/kg), Ketamine (25 mg/kg) and Xylazine (25 mg/kg) at the ratio 1:1:1 with 0.1 ml/100 g body weight. The stomach of the experimental animal exposes by laparotomy with anesthetized. 0.03 ml 20 % acetic acid injects into the subserosal layer of the glandular part of the stomach with the help of a micro-syringe. The animals should be free from food for 8-9 hours. The animals receive H. pylori inoculums and drugs. Rats inoculated with 1 ml of pathogenic serum of H. pylori suspended in MHB after 24 hours acetic acid induce ulceration.[12, 13] After treatment, the animals are sacrificed after 4 hours of administration of the test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The area and diameter of the ulcer measure with help of a ruler. The ulcer score calculates on the base of the severity of gastric ulcer lesion. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.[14]

Acetic Acid Induce Gastric Ulcers

This model is used to develop chronic gastric ulcers. Acetic acid interferes with the pH of gastric fluid. This model suitable for screening the effect of potential drugs and the evaluation of test drugs that use for healing chronic ulcers. This model also uses anti-secretary and cytoprotective evaluation [11]. Different animals maybe use for this experimental model.[15]

Procedure

Albino rats (150-200 g) use for experimental work in his model. The experimental animals are anesthetized with a general anesthetic such as pentobarbitone (35 mg/kg). When an animal is completely anesthetized then open the abdomen and stomach is visualized. 50 % acetic acid solution (0.06 ml/rat) is dropped into the tube that has a 6 mm diameter is tightly put on the gastric (stomach) wall and allowed to remain for only 1 minute. The abdomen of the rat folds in two layers after removal of acid solution and animals are put in the cage for normal fed. After 4 hours petition of acetic acid the test and standard drug administered through oral route for 9 days after the induction of gastric (peptic) ulcers.[16] The last test and standard-dose administered 10th day and after 18 hours the animals anesthetized and sacrificed. The abdomen open and dissected its stomach and determine the ulcer index and ulcer scores.[17]

Cysteamine-induced duodenal ulcers

It is a model that is used to induce duodenal ulcers in rats. This model firstly describes by Selye and Szabo. This model widely uses as induction of duodenal ulcer for the screening of antiulcer drugs.[18-20]

Principle and Mechanism

Cysteamine stimulates gastric acid secretion and inhibits the production of alkaline mucous from Brunner's gland.[21]

Procedure

Firstly, animals select for experimental work. Male Wistar rats (200-250 g) use for this model.[21] All animals free from food for 24 hours but not water. The experimental animals were divided into groups. Group first administers only normal saline solution and group second administers standard (gastro-protecting) drug. The cystamine hydrochloride (450 mg/kg) administers to the animals.[22] Animals are sacrificed after 4 hours of administration of the test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.

Diethyldithiocarbamate- (DDC)-induced Ulcers

The diethyldithiocarbamate model is used to produce lesions in the stomach of rats. This model also uses for the screening of antioxidant activity.

Principle and Mechanism

Diethyldithiocarbamate generates free radicals such as superoxide and hydroxyl radicals that suppressed gastric mucosal copper-zinc superoxide dismutase (Cu, Zn-SOD) activity result in gastric lesions. Diethyldithiocarbamate also decreases blood flow in gastric mucosal that causes gastric ulcers.[23]

Procedure

Animals select for experiments and divide them into groups. All animals fast for 24 hours. Animals should free from water four two hours. Acute glandular lesions produced by subcutaneous administration of 1 ml of diethyldithiocarbamate in saline (800 mg/kg body weight) followed by 1 ml oral dose of 0.1n HCl. Test drug administers to the animals after 2 hours. [24, 25] Animals are sacrificed after 4 hours of administration of the test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.[26, 27]

Ethanol Induced Ulcers

Absolute ethanol uses for induction gastric ulcers in the experimental animals. Alcohol penetrate the mucous of the stomach, therefore, cause gastric ulcers.

Principle and Mechanism

Ethanol disturbs the gastric secretion through gastric mucous depletion, damage the mucosa, alteration in permeability and generation of free radicals. When ethanol metabolizes then produce free radicals such as hydroperoxy free radicals and superoxide free radicals.[28] Alcohol is responsible for penetrating the gastric mucosa

therefore damage the cells and increase the permeability to sodium and water. Intracellular calcium also accumulates and causes pathogenesis of gastric injury that is responsible for the death of cells and exfoliation of the surface of gastric layers.[29] Alcohol also increases the levels of malondialdehyde that is responsible for increase lipid peroxidation.[30]

Procedure

Generally experimental rats (150-200 g) use for ethanol induce ulcer model. Firstly, weigh either sex rats then divide into groups. Animals should fast for 24 hours with free access to water. The animals are administered test drug or standard drugs. After 1 hour 1 ml absolute ethanol (99.80 %) administer orally to the experimental rats. The rats are anesthetized with ether 1 hour after alcohol dose.[31] When animals anesthetized then dissected their stomach with greater curvature and calculates estimate gastric contents, pH of contents, total acidity and also calculates ulcer index.[32] Finally, stomach tissues prepare for histopathology and evaluate the histochemical section by light microscope. For histopathology, a small fragment of the stomach wall of each animal is fixed with 10 % formalin buffer solution followed by tissue dehydration with xylene and alcohol. The section of the stomach wall is embedded in paraffin wax and sectioned (3-5 µm) slide before staining. Haematoxylin and eosin dye used for staining. The histochemical section was observed under a light microscope.[33]

Ferrous iron-ascorbic acid-induced gastric ulcers

In this model the solution of ascorbic acid and ferrous iron use for the induction of gastric ulcer with direct local injection at the gastric wall.[34]

Principle and Mechanism

In this model, the solution of ferrous iron and ascorbic acid interfere in the lipid peroxidation, therefore, generate free radicals (oxygen radicals) that causes ulceration in the stomach wall.[35]

Procedure

Male albino rats select for this experimental work. The weight of the animal should not less than 150 g. The animals divide into groups. All animals free from fed for 18 hours before start the experimental work but not free from water. 25 µl of the drug dissolved in the normal saline and prepare a solution. This solution injects into the submucosa anterior wall of the stomach with the help of a microsyringe (Naito, et al 1995). Animals are sacrificed after 4 hours of administration of the test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.

Histamine Induced Gastric Ulcers

Histamine is amine of tissue that is responsible for the secretion of gastric acid with disturbance of gastric mucosa. Generally, histamine present in the animal within storage granules of mast cells. Histamine rapidly stimulates the secretion of (HCl) hydrochloric acid with disturb in the gastric mucosa.[36] Histamine not only increases secretion of hydrochloric acid in animals but also results in an abnormality in the gastric motility, decrease mucous production, mucosa and microcirculation in the stomach.[37]

Principle and Mechanism

Mast cells present in the wall of the stomach that secretes histamine. Histamine binds with histamine receptor (H_2) are present on the surface of parietal cells. Histamine stimulates adenyl cyclase enzyme that converts adenosine triphosphate into c-AMP, which in turn activates the membrane proton pump (H^+K^+ATPase) then stimulates the secretion of hydrochloric acid.[36 38]

Procedure

Mostly guinea pigs (300 – 450 gm) use as an experimental animal for this model. The animal does not administer food for 48 hours before the experiment or administration of histamine. 1 ml histamine acid phosphate solution (50mg/ml/ip) administer to the guinea pig and after one hour administer test drug dose to the animal. After four hours of histamine treatment, the animal anesthetized and dissected out the stomach. All content of stomach estimates and the score of ulceration compared with control animals.[39]

Hydrochloric Acid Induce Gastric Ulcers

Hydrochloric acid induces peptic ulcers with a direct increase in the acidity in the stomach. This animal model uses for experimental rats and mice.[40]

Procedure

Experiment rats (weight about 150-180 gm) may be used for this experiment. Rats divide into groups and deprived of food for 24 h in a cage. Test, control and standard drug administer to the rats after 30 minutes HCl with ethanol (98% ethanol containing 150 mM HCl) the dose 5 ml/kg administer to each animal.[41] After 1 hour each animal anesthetized with a general anesthetic such as ether. When an animal completely anesthetized the open its abdomen and dissects the stomach with the help of greater curvature. The inner surface of the stomach washes with normal saline solution and observed the ulceration.[42] The gastric tissue fixes for 24 hours in 10% formalin for the examination of histopathological study. The section of the stomach wall is embedded in paraffin wax and sectioned (3-5 µm) slide before staining. Hematoxylin and eosin dye used for staining. The histochemical section was observed under a light microscope.

Ischemia-reperfusion- (I-R-) induced gastric ulcers

Ischemia-reperfusion model generally uses for the induction of gastric ulcers. The mucosa of the gastrointestinal tract is sensitive to ischemia.[43]

Principle and mechanism

Ischemia-reperfusion is responsible for the generation of free radicals which results in the development of ulceration and erosion in the gastric mucosa.[44]

Procedure

Firstly, experimental animals select for experiments and divide them into groups. The animals should be fast for 20 hours before the experiment. Animal's anesthetized with a mixture of xylazine and ketamine (15+60 mg/kg). The ends of the stomach both esophageal and pyloric are bind with bull god clips using laparotomy. The celiac artery carefully isolates from its adjacent tissues. The celiac artery ligates with a ligature for 30 minutes to induce ischemia and the ligature is removed to allow reperfusion for 3 hours.[45] The test drug administered intraperitoneal to animals.[46] Animals are sacrificed after 4 hours of administration of the test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index.[47] The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.[46]

Methylene Blue Induced Ulcers

Methyl blue is a synthetic drug that is used to induce lesions in the gastric mucosa. Methylene blue generally uses for screenings of antiulcer drugs.

Principle and mechanism

Methyl blue activate H+/K+-ATP-ase therefore the secretion of hydrochloric acid in the stomach increase and causes gastric lesions. Methyl blue also generates free radicals such as superoxide dismutase that cause oxidative stress and result in gastric ulcers. Methyl blue also interferes with the blood supply that causes acidity. Methyl blue show affinity for (M receptors) muscarinic or acetylcholine receptors and inhibit the activity of cholinesterase.[48]

Procedure

Generally, experimental rats use for this experiment. Adults' albino rats (weight 150-250 g) select and divides into groups. Test dose and standard drug dose (such as ranitidine 50 mg/kg and omeprazole 200 microgram/ml) administered to the animals. The animals fast for 24 hours and methyl blue (125 mg/kg) is administered to the animal through an oral route. Animals are sacrificed after 4 hours of administration of methyl blue. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.[49]

NSAIDs Induced Peptic Ulcers

Non-Steroidal Anti-inflammatory Drugs such as Aspirin, Ibuprofen and Indomethacin commonly used for produced peptic ulcers in experimental animals. It is the most common experimental animal model use for the anti-ulcer activity.

Principle and Mechanism

Nonsteroidal Anti-inflammatory Drugs (NSAIDs) are considered one of the most uses medications for patients for the treatment of pain and inflammation. NSAIDs administration displays the main cause of peptic ulcers.[50] NSAIDs cause gastric ulcers by inhibiting prostaglandin secretion, inhibiting the formation of lipid peroxidation and generate a reactive oxygen species (ROS).[51] Indomethacin promotes apoptosis and necrosis of cells of the stomach.[52] NSAIDs block the activity of COX-I and COX-II (Cyclooxygenase enzyme) hence leading to inhibit mucosal blood flow, inhibit mucous and bicarbonate secretion, interfere in platelets aggregation and disturbing in microvascular structure.[53] Indomethacin induces gastric motility, inhibits mucous secretion and bicarbonate and disrupting production of nitric oxide production in stomach tissues.[54] it was found in various studies that indomethacin inhibits the release of protective factors, inhibiting antioxidant parameters while stimulating oxidant parameters.[55]

Procedure

Indomethacin and aspirin are the most common frequently use for the induction of gastric ulcers. The experimental animals (rats) fasted for about 36 hours before start the experiment. The experimental animal's treats with test drug 30 minutes before the administration of ulcer induce drugs like aspirin (200 mg/kg suspension in carboxymethyl cellulose) two-dose at an interval of 15 hours, after 6 hrs animals sacrificed.[56] Phenylbutazone administers 100 mg/kg to the experimental animal at the 15 hrs dose interval and sacrificed after 6 hours. The dose of reserpine is 5 mg/ kg for the experimental animal after administration 24 hours of animal scarifies. The dose of indomethacin is 10 mg/kg for the animal after 36 hours of fasting and after the treated dose, 15 hours animal scarifies.[57]

Pylorus Ligation Model

The Pylorus ligation model is a surgical procedure that is used for the induction of gastric ulcers. In this model, stomach pylorus ligates with the help of surgery without damage blood vessels.

Principle and Mechanism

When the lower part of the stomach ligates, then automatic digestion starts and breakdown of the stomach wall because gastric juice such as pepsin and gastric acid secretes for digestion [58]. When pylorus ligated then the blood supply of the stomach disturbs and free radicals generated that are responsible for gastric juice production therefore induced gastric ulcers through this model.[596061]

Procedure

In this model, mainly rodent animal (Rat 140-165 gm) selects for the experiment. Firstly administered the

anesthesia to the animal then open the abdomen and ligate its stomach without damage blood vessels and then sutured it.[62] Testing a drug or agent administered to the animal after operation within two days. Dissected the stomach after 18 hours and collect its content for estimation of acidity and pH measurement. Check the ulcer at the wall of the stomach of the treated group and standard drug and compare gastric volume, acidity, pH and ulcer index with the control group.[63]

Reserpine Induced Gastric Ulcers

Reserpine is an active chemical that is derived from the roots of the medicinal plant *Rauwolfia serpentine*. This chemical is an alkaloid in nature that has antipsychotic and antihypertensive activity. reserpine is used for induction the gastric ulcer in experimental animals such as mice and rats.[64]

Principle and Mechanism

Reserpine produces the gastric ulcer due to the degranulation of mast cells therefore increase the secretion of gastric acid by sympathetic activation.[65] It was observed that reserpine causes gastric ulcer with disturbing in the serotonin, catecholamine and histamine store at (CNS) central nervous nervous system and peripheral nervous system.[66] Reserpine also generates free radicals and inhibits the production of prostaglandins.[67] Reserpine disturbs the blood supply in the stomach mucosa and alters the gastric motility.[68]

Procedure

Mice selected for this experiment and it was divided into groups.[69] All animals are fasts for 48 hours after the test drug administers intraperitoneally to the animals. After 1 hour the ulcer induces agent reserpine (10 mg/kg) administers intraperitoneally to the animals. After 4 hours all animals anesthetized with ether and then scarify and dissects their stomach. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.[70]

Serotonin Induced Gastric Ulcers

Serotonin uses for the induction of gastric ulcers in the experimental rats. This model use screening of antiulcer drugs.

Principle and Mechanism

Serotonin reduces the blood flow in the gastric mucosa and acts as a vasoconstrictor therefore induces the ulcer in the stomach.[71]

Procedure

Firstly, albino rats select for experiments and divide them into the group. All rats free from food for 24 hours but not water and it confirms that their stomachs are empty. Before 2 hours of ulcer, induction stops the receiving of water to rats.[72] The control group administers only a normal saline solution.[73] A single dose of serotonin (0.5 mL of 50 mg/kg) administers by subcutaneous injection to the rats. Serotonin administers to the rats by intra-gastric intubation with the help of an orogastric cannula. Animals are sacrificed after 6 hours of administration of test drug.[74] The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.

Water- Immersion Stress-Induced Ulcers

This model was used for the develop gastric ulcer in rats. The technique of this model was developed by Hanson and Brodie.[75] Levine developed ordinary water immersion or cold-water method.[76]

Principle and Mechanism

This model induces gastric ulcers due to disturbance in the gastric mucosa causing an increase in the secretion of histamine therefore increase the acid secretion and inhibit mucous production.[77] Water immersion stress induces the motility of gastric folds of the stomach and disturbs the blood supply.[78]

Procedure

All animals were divided into groups and fasted for 24 hours with free from food and drinking water before starting the experiment. Each rat restrained individually in a cage and immersed up to its xiphoid in temperature-controlled water (about 23℃) for 10 hours. After this, the animals anesthetized and the stomach of each animal ligate at pylorus and cardia and fixed by paraformaldehyde for 24 hours. Then the stomach opens with the help of greater curvature and dissects it. The dissect stomach wash with cold saline solution to remove the gastric contents. The stomach flattened and captures its photographs. After this calculate ulcer index, ulcer scores and prepare the slide for pathophysiology.[79]

PARAMETERS TO BE CALCULATED

There are seven parameters i.e., pH, Volume of gastric contents, Total and Free Acidity, Lipid Peroxidation, ulcer index, % protection ratio and % curative ratio, calculated by using the method described by different scientist to evaluate the anti-ulcer activity of the drug in *in-vivo* models.

pH

pH of gastric content determines by dipping the electrode of the pH meter in a beaker containing gastric contents.[80] [81]

The volume of gastric contents

The volume of gastric contents measures pouring gastric Contents carefully in the graduated cylinder.[82]

Total and Free Acidity

Collect one mL of gastric content and centrifuge it and filter for titration against 0.1 sodium hydroxide solution using

the Topfers reagent. This reagent uses as an indicator for the determination of free acidity. 1% solution of phenolphthalein indicator use for confirmed total acidity. The sum of two titrations will be total acidity.[83]

Lipid Peroxidation

The glandular part of the stomach tissue will be homogenized in trichloroacetic acid (TCA) and the homogenate use to estimate malondialdehyde. Briefly, lipid peroxidation will be induced by adding ferric chloride (10 1d. 400mM) and 1-ascorbic acid (l0d, 400mM) to a mixture containing stomach homogenate (0.3 ml) in phosphate buffer solution (5 ml pH 7.4. 0.2 M). After incubation for 1 h at 37 °C. the reaction will be stopped by adding hydrochloric acid (2 ml. 0.25 N) containing trichloroacetic acid (1 ml. 15% w/v) and thiobarbituric acid (0.5 ml. 0.375% w/v) boiled for IS mm. cooled, centrifuged and absorbance of the supernatant % will be measured at 532 nm.[84]

Scoring of ulcers based on ulcer severity.

For calculating ulcer following may be considered:[13]

Score	Ulcer severity
0	No lesions
1	mucosal edema
2	1-5 small lesions (1-2 mm in size)
3	> 5 small or intermediate (3-4 mm in size) lesions
4	≥ 2 intermediate lesions or 1 gross (> 4 mm in size) lesion
5	Perforated ulcers

Calculation of Ulcer Index (UI) based on ulcer score

By using the ulcer score as described above, the ulcer index can be calculated as follows:

$$\text{Ulcer Index (UI)} = \frac{\text{Total ulcer score}}{\text{Number of animals ulcerated}}$$

Calculation of % protection ratio and % curative ratio by using the Ulcer Index

The following formula may be used for the calculation of percentage protection and percentage curative ratio [85]

$$\text{\% protection ration} = \frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated}} - \frac{\text{UI of drug pre treated group}}{\text{UI of ulcerogen treated}}$$

$$\text{\% Curative Ratio} = \frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated}} - \frac{\text{UI of drug treated group}}{\text{UI of ulcerogen treated}}$$

CONCLUSION

It is undeniable that animal models have led to human science. Without a high fidelity, highly reproducible model, with the added advantage of avoiding potential human damage, many modern developments would simply not have been made possible. However, a closer look at the present environment poses concerns. We must reexamine the use of sentient animals in human research with the implementation of alternatives such as simulation, with an eye to reduction, enhancement, and finally replacement if possible.

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