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EVALUATION OF ANTI-ULCER ACTIVITY OF INDAZOLE AND ITS DERIVATIVES

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ABSTRACT

The biological activities of the heterocyclic compound, indazole, including anti-inflammatory, antimicrobial, anti-HIV, anticancer, hypoglycaemic, antiprotozoal, antihypertensive, and other activities. From the observed biological activities of the indazole moiety, it is concluded that the medicinal properties of indazole have to be explored in the near future for the treatment of various pathological conditions. The present study is concerned with exploring the potential pharmacological actions of indazole and its derivatives. The main focus is on their role in ulcer protective effect against pylorus ligation-induced gastric ulceration, Swimming stress induced ulcers, Aspirin induced ulcer in rat modal. The present study identified that, all investigated compounds may prevention of pylorus ligation-induced gastric ulceration, Swimming stress induced ulcers, Aspirin induced ulcer. They can be combined with any of the currently used NSAIDS to improve its therapeutic efficacy and at the same time to blunt /offset gastric ulceration, the most feared adverse effect of NSAID. All tested compounds have ulcer protective effect induced by pylorus ligation, Swimming stress, aspirin. 6-Nitroindazole have better activity compared with Indazole and 5-Amino indazole.

KEYWORDS: Indazole, 5-Amino indazole, 6-Nitroindazole, Anti-ulcer activity, NSAID.

INTRODUCTION

Peptic ulcer is a public health problem with high rate of morbidity and substantial mortality and has become the focus of experimental and clinical investigations, mainly due to its high prevalence in the global population.[1] Peptic ulcers are usually aggravated by an imbalance between destructive and defensive factors in the stomach.[2] The endogenous destructive factors in the stomach are HCl, pepsin, biliary reflux, lipid peroxidation, and the formation of reactive oxygen species (ROS) and the exogenous factors are excessive use of ethanol, indiscriminate use of nonsteroidal anti-inflammatory drugs (NSAID), stress, smoking, and infection by *Helicobacter pylori* bacteria.[3–6] The defensive factors are mucus-bicarbonate barrier, mucin secretion, surface phospholipids, prostaglandins (PGs), nitric oxide (NO), mucosal blood flow, cell renewal, growth factors, and antioxidant enzymes.[2,4,5] Oxidative stress, present in the process of gastric ulceration, increases the formation of ROS that can disrupt epithelial cell integrity. An excess production of ROS metabolites may overwhelm the endogenous antioxidants.[7] In addition, ROS accumulates neutrophils in the tissues of the mucosa during gastric ulceration. Studies have shown that proinflammatory cytokines induce the activation of neutrophils and are strong contributors to the of ulcer damage.[8,9] Effective therapies for peptic ulcers use alternatives that control acidic hypersecretion and its direct effects on the gastric mucosa. The two main classes of drugs used to treat acid-related disorders include proton pump inhibitors (PPI) that inhibit the hydrogen pump in the parietal cell directly, independently of any membrane receptor stimulation, and histamine type 2 receptor antagonists (H2RAs), which block the histamine receptor on parietal cells thereby reducing hydrogen ion release.[10] PPI is among the most prescribed drugs in the world; however, it may lead to the development of parietal cell hyperplasia of the gastric glands.[11] Long-term use of H2RAs is associated with the development of undesirable effects such as gynecomastia and galactorrhea as well as alteration of the bacterial flora of the gastrointestinal tract.[12]

Indazole was first defined by scientist Emil fisher as a “pyrazole ring fused with the benzene ring”. it is extensively studied due to its interesting chemical and biological properties. Indazole belongs to the azole’s family containing carbon, hydrogen and nitrogen atoms. indazoles are also called as benzpyrazole or isoindazolone heterocyclic organic compounds, possessing two nitrogen atoms. indazole having ten p-electron aromatic heterocyclicsystems as like that of pyrazole molecule and indazoles resembles with pyridine and pyrrole. The structure of indazole in cylindrical

bonds.[13] The first compound known to contain the indazole ring system was indazolone and its preparation, by heating *o*-hydrazinobenzoic acid was reported in 1880 by Fischer. Indazole itself was first prepared a few years later by Fischer and Kuzel. Since then, several approaches for the preparation of indazole and its derivatives have been developed. The most frequently used method is the use of *o*-disubstituted benzene derivatives, where the twosubstituent react to form a pyrazole ring.[14]

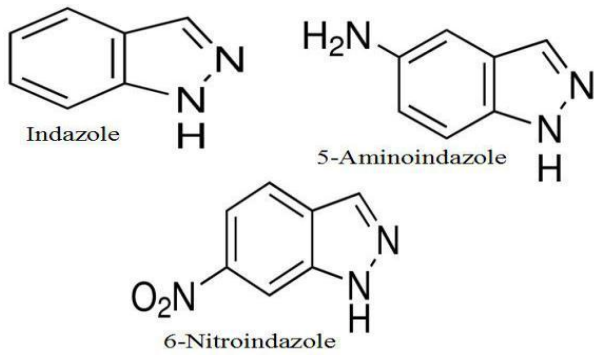

Figure 1: Structures of indazole and its derivatives.

MATERIALS AND METHODS

Indazole and its derivatives: Drugs used in this study are; indazole,5 –aminoindazole and 6 –were selected for the study and these were purchased from Sigma Aldrich, USA. The test compounds were prepared as a fine suspension in 0.5% carboxyl methyl cellulose (CMC) and injected i.p. different doses for different test.

Animals: Wistar rats weighing 25 – 30g were used in the present study. The animals had free access to food and water and maintained at 24 ± 1° C temperature with 12h day/ 12h night cycle. All the experiments were carried out between 09.00 and 13.00 hours to avoid circadian variation. The experiments were carried out in Jeeva life Sciences (Jeeva life Sciences have in house breeding). The experimental protocol was approved by the institutional animal ethical committee (CPCSEA/IAEC/JLS/15/05/21/06). In all the experimental studies each group consisted of five animals.

EXPERIMENTAL PROCEDURE

Pharmacological studies

Pylorus ligation induced gastric ulceration in rats

Overnight fasted rats were anaesthetized with ether. Surgical incision was given in abdomen below the sternum. Stomach was exposed and thread was tied around the pyloric sphincter and a tight knot was applied in such a manner that blood vessels were spared. The abdomen wall was closed by putting sutures. Colloidion was applied over the wound. Cimetidine (10 mg/kg, p.o.) was given 15 min prior to surgery as a standard. After 4 hr, animals were sacrificed by decapitation. Abdomen was opened and tied the esophageal end of the stomach. Cut was given and the entire stomach was removed from body of the animal. A small cut was given to the pyloric region just above the knot. Gastric contents were collected in graduated centrifuge tube and were centrifuged at 1000 rpm for 10 min. Volume of supernatant was noted as volume of acid secreted. Thereafter, 1 mL of supernatant was pipetted out and it was diluted up to 10 mL with distilled water. The pH of this solution was noted with the help of pH meter. thereafter the solution was titrated against 0.01 N NaOH solution using Topfer's reagent as indicator, which is dimethyl-amino-azo-benzene with phenolphthalein and used for detection and estimation of hydrochloric acid & total acidity in gastric fluids. At the end point, the solution turns to orange color. The volume of NaOH consumed noted which corresponds to the free acidity. Titration was carried out further till the solution regains pink color. Again, the total volume of NaOH will be noted which corresponds to the total acidity. Acidity (mEq/ L/100g) will be expressed.[15]

Swimming stress induced ulcers

Wistar rats fasted for 24-36 hours were forced to swim inside the vertical cylinders (height 30 cm, diameter 15 cm) containing water up to 15 cm height, maintained at 23℃. Three hours after the stress, they were removed from the cylinders and sacrificed by a blow on the head. The ulcer index and mucin content were determined as described above. In this test diazepam served as standard (2 mg/kg; p.o.). Test drugs were administered 30 min prior to stress.[15]

NSAID induced gastric ulcers

Gastric ulceration in rats was induced by drugs and the ability of several agents to either protect against or aggravate this ulceration was observed. The compounds under investigation were administered 30 min to 1 h before Aspirin (20 mg/kg, p.o.) administration. The animals were sacrificed after 4 h and the stomachs were examined for the presence of mucosal lesions. The ulcer index and mucin content were determined as described above.[15]

EVALUATION OF PARAMETERS

Gastric Juice Volume

The stomach was excised carefully, opened along the greater curvature and the gastric contents were removed. The gastric contents were collected in a graduated tube and centrifuged at 2000 rpm for 10mins. The supernatant liquid was collected and expressed as ml/100gm body weight.

Ulcer Score, Ulcer Index and % Inhibition

After rats were sacrificed, the stomach of a rat was opened along the greater curvature, washed it slowly under running tap water and put it on the slide and observed under 10X magnification for ulcer. Score of ulcers was determined as:

0 = Normal colored stomach, 0.5 = Red coloration, 1 = Spot ulcers, 1.5 = Hemorrhagic streaks, 2 = Ulcers ≥ 3

but ≤ 5, 3 = Ulcers > 5

The mean ulcer score for each animal was described as Ulcer index. The formula for ulcer index is as follows:

$$UI = UN + US + UP \times 10^{-1}$$

Were
UI = Ulcer index,
UN = Average number of ulcers as per animal,
US = Average number of severity score in animals,
UP = Percentage of animals with ulcers.

Percentage of ulcer inhibition was calculated as:

$$\% \text{ Inhibition of ulcer} = \frac{\text{Ulcer index control} - \text{Ulcer Index test}}{\text{Ulcer index control}} \times 100$$

Free Acidity and Total Acidity

The content of gastric juice was centrifuged at 1000rpm for 10 mins. Pipette out 1ml of supernatant liquid and dilute it to 10ml of distilled water. Note the p^H of the solution with the help of p^H meter. Titrate the solution against 0.01N NaOH solution using topfers reagent as an indicator. Titrate to end point when the solution turns to orange colour. Note the quantity of NaOH that corresponds to free acidity. Titrate further till the solution regains its pink colour by using phenolphthalein as an indicator. Note the total volume of NaOH, that represents total acidity. It can be expressed as mEq/l/100g. Following formula was used to calculate acidity:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality}}{\text{mEq/l/100g}} \times 100$$

$$0.1$$

Gastric Juice p^H

The p^H of gastric juice was recorded with the help of digital p^H meter.

Total Acid Output

Total acid output = It is expressed as μEqL^{-1}/100g body weight

Statistics analysis

All data were expressed mean±SEM. For comparison amongst groups post hoc one-way ANOVA was performed. P value less than 5% (P<0.05) was considered to be statistically significant.

RESULTS AND DISCUSSION

Effect of Indazole and its derivatives on pylorus ligation-induced gastric ulceration

Significant (P<0.05) change in observed parameters i.e. volume of gastric secretion, free acidity, total acidity, ulcer index and mucin content were observed in vehicle control group as compared to sham control, indicating significant ulceration, due to accumulation of gastric contents in ligated animals. Treatment with Indazole and its derivatives significantly (P<0.05) reduced gastric volume, free acidity, total acidity, ulcer index and increased the mucin content. Treatment with 6-Nitroindazole significantly (P<0.05) reduced the gastric pH. Similar protection was also observed after treatment with 5- amino indazole. However, the 6 Nitro indazole showed better protection as compared to 5-aminoindazole, which was comparable to cimetidine treatment. (Table 1).

Swimming stress induced ulcers

In stress induced ulcer test significant ulceration was observed in vehicle control group, indicated by ulcer index and decreased mucin content. Treatment with the 6-nitroindazole significantly (P<0.05) decreased the ulcer index and increased the mucin content. Similar protection was also observed after treatment with diazepam (Table 2).

NSAID induced gastric ulcers

dministration of Aspirin resulted in a significant ulceration in vehicle control group indicated by increased ulcer index up to 5.12± 0.13 and decreased mucin content of 28.8±0.77. Treatment with 6-Nitroindazole significantly (P<0.05) decreased the ulcer index and increased the mucin content (Table 3).

Table 1: Effect of Indazole and its derivatives on pylorus ligation induced gastric ulceration in rats (n=5).

Group name	Volumeof gastric secretion(mL/100g)	pH	Free acidity((meq/L/100g)	Total acidity((meq/L/100g)	Ulcer index	Mucin content
Sham control	-	-	-	-	-	43.20±1.03
Vehicle Control	5.18±0.07*	2.38±0.06	66.12±0.06*	197.20±0.34*	5.26±0.05*	25.00±0.06*
Indazole (100mg/kg)	4.40±0.03	2.58±0.04	62.00±0.04	192.20±0.44	5.02±0.07	24.20±0.68
5-aminoindazole (100mg/kg)	4.16±0.04	2.64±0.05	58.92±0.06	188.80±0.34	4.92±0.09	30.40±0.46
6-Nitro indazole (100mg/kg)	3.90±0.04	2.40±0.04	58.12±0.04	184.60±0.36	4.60±0.07	30.80±0.46
CMD 10 mg/kg	1.66±0.05a	5.40±0.03a	31.12±0.07a	94.20±0.66a	1.56±0.06a	39.00±0.63a

*P<0.05 vs. sham control; aP<0.05 vs. control. CMD: Cimetidine,

Table 2: Effect of Indazole and its derivatives on pylorus ligation on swimming stress induced gastric ulceration model (n=5).

Group name	Sham control	Vehicle control	Indazole (100mg/kg)	5-aminoindazole (100mg/kg)	6-Nitro indazole(100mg/kg)	DZP
Ulcer index	-	3.94±0.05	2.86±0.04	1.90±0.03a	1.82±0.06a	1.45±0.05a
Mucin content	43.20±1.03	27.40±0.83	28.80±0.02	36.40±0.78	37.50±0.06	39.40±0.63

P<0.05 vs. control. DZP: Diazepam.

Table 3: Effect of Effect of Indazole and its derivatives on Aspirin induced ulcer activity model (n=5).

Group name	Sham control	Vehicle control	Indazole(100mg/kg)	5-aminoindazole (100mg/kg)	6-Nitro indazole (100mg/kg)	CMD
Ulcer index	-	5.12±0.13	4.92±0.13	3.02±0.03a	2.87±0.06a	1.10±0.05a
Mucin content	43.20±1.03	28.80±0.77	30.10±0.53	37.60±0.92a	38.10±0.75a	39.20±0.61a

P<0.05 vs. control, CMD-10: Cimetidine

Physiological stressors are known to produce gastric ulcers by complex central and peripheral mechanism. The present study investigated the effect of Indazole, 5-Aminoindazole,6-Nitroindazole on pylorus ligation, swimming stress and indomethacin -induced gastric ulcers. Among all 6-Nitroindazole showed significant protection. However, 6-Nitroindazole showed maximum activity therefore was only used in other animal ulcer models.

Pylorus ligation-induced ulcers are thought to be caused by increased presence of acid and pepsin in the stomach. This model mimics the most common clinical condition of chronic constipation leading to gastritis and ulceration because of low gastric motility which leads to prolonged gastric acid secretion thus increasing offence. Further, the essential criteria, which determine the status of mucosal defence barrier against the offensive assault of acid-pepsin is the quality and quantity of gastric mucus secretion. Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms including lessening stomach wall friction during peristalsis and acting as an effective barrier to the back diffusion of hydrogen ions. As significant increases in mucin content have also been observed with 6-Nitroindazole reduced the pylorus ligation-induced ulceration suggesting their mucosal barrier strengthening ability in addition to antisecretory activity.

Several physical and psychological factors are involved in the genesis of stress- induced ulcers Increase in gastric motility, vagal over activity, mast cell degranulation, decreased mucosal blood flow, and decreased prostaglandin synthesis are some of the important factors among them.

The 6-Nitroindazole treatment resulted in protection of stress induced ulcers, indicating its antistress potential. However, further studies in future will provide more information to understand its exact mechanism of antistress effect.

Non-steroidal anti-inflammatory drugs like, aspirin, indomethacin etc. are known to induce ulcers during the anti-inflammatory therapy. The same has been implemented in laboratory to induce ulcers in experimental animals. These drugs induce ulcers by inhibition of prostaglandin synthesis through the cycloxygenase pathway. Inhibition of prostaglandin production results in increased acid production and decreased cytoprotective mucus formation, which can lead to induce gastrointestinal ulcer. ROS also has an important role in the mucosal damages caused by Aspirin and other agents. SOD, GST and CAT are some of the antioxidant enzymatic defense mechanism. It has been reported that SOD activity in rat stomach tissues is decreased by non-steroidal anti-inflammatory drugs. The 6-Nitroindazole showed marked gastroprotective properties as evidenced by its significant inhibition of the formation of gastric lesions (in terms of length and number) induced by Aspirin.

CONCLUSION

The main aim of the present study was to investigate a group of Indazoles (Indazole,5-aminoindazole, and 6-nitroindazole) for their possible role, anti-ulcer has been investigated.

The present study identified that, all investigated compounds may prevention of pylorus ligation-induced gastric ulceration, Swimming stress induced ulcers, Aspirin induced ulcer. They can be combined with any of the currently used NSAIDS to improve its therapeutic efficacy and at the same time to blunt /offset gastric ulceration, the most feared adverse effect of NSAID.

All tested compounds have ulcer protective effect induced by pylorus ligation, Swimming stress, aspirin. 6-Nitroindazole have better activity compared with Indazole and 5-Amino indazole.

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