DISCUSSION
5. DISCUSSION

5.1. Identification of *H. pylori*

A variety of host and bacterial factors contribute to the pathogenesis of gastrointestinal diseases resulting from *H. pylori* infection. It secretes various factors like urease, catalase, mucinase, lipase, hemolysin and alkaline phosphatase that decrease viscosity of mucus. The production of catalase protects the bacteria against the toxic effects of reactive oxygen metabolites formed in neutrophils from hydrogen peroxide. The multiple polar flagella permit them to penetrate the mucus layer. Adherence of *H. pylori* to gastric epithelial cells and vacuolating cytotoxin are the virulence factors, as they are associated with degenerative changes in the epithelial cells (Aroori, 2001). Rapid urease test is one of the invasive tests and considered as the gold standard test for *H. pylori* infection. It is based on the principle that abundant urease enzyme produced by *H. pylori* hydrolyses urea to ammonia. The consequent rise in the pH of the medium is detected by phenol red indicator.

\[
\text{NH}_2\text{-CO-NH}_2 + 2\text{H}_2\text{O} + \text{H}^+ \rightarrow 2\text{NH}_4^+ + \text{HCO}_3^-
\]

In that case RUT yields positive result as *H. pylori* get sufficient time to multiply in the urea broth. This clarifies the reason for getting 6 samples of all *H. pylori* groups positives by RUT in our study. Interestingly an inverse relationship exists between the prevalence of the infection and socio-economic factors with higher infection rates in developing countries (Veldhuyzen van zanten, 1995; Webb et al., 1994).

The CLO test is done in clinical practice for the biopsies of gastric mucosa placed in a gel containing urea, and a subsequent ammonia production causes a pH change, which is observed as a color change. The CLO test depends greatly on the pH of the media and the amount of the urea in the medium. These factors may vary in different products and thereby influence the results obtained with other tests. CLO test is one of
the best identification for the \textit{H. pylori} (Andersen et al., 1998; Thijs et al., 1996).

Serology by ELISA has been used to detect the \textit{H. pylori} infections in patients with the gastritistic ulcer which is quite costly. A duration of 3 to 5 hours is needed to complete ELISA test. Moreover while performing ELISA, a sample of tests is put up at one time keeping the economic factor in mind as we need to run positive and negative controls simultaneously. This is the test which confirms the infections of \textit{H. pylori} with absolute accuracy. Serological approaches are non invasive tools widely used for determining \textit{H. pylori} infection and ELISA assays are more popular than other approaches among clinicians and practicality of this technique and their high sensitivity. Evaluation of specificity and sensitivity of these tests results in disagreeing and even contradicting results (Miwa et al., 2000).

In this study, we conformed the clinical isolates in gastric biopsy a specimen from patients infected with \textit{H. pylori} was based on positive culture of \textit{H. pylori} or urea test of biopsies. Three biopsies were taken from the gastritis patients. Apart from having support of clinical evidences of gastric ulcer, all the isolates HP-B\textsubscript{1}, HP-B\textsubscript{2}, and HP-B\textsubscript{3} were found to be positive for the above mentioned test. All the \textit{H. pylori} strains were conformed not only on the basis of urease test but were also conformed by CLO test as well as ELISA test. These positively identified cultures were taken for further studies and marked as HP-B\textsubscript{1}, HP-B\textsubscript{2}, HP-B\textsubscript{3} and HP-B\textsubscript{4} respectively.

5.2. Kinetic growth study

All \textit{H. pylori} groups were grown in BHI and MH broth and agar plates for isolation and only BHI was taken for kinetic growth study. The concentrations of cells achieved in this study are satisfactory for achieving a large quantity of cells using inexpensive and readily available equipment. Other studies have achieved higher peak concentration of cell using kinetic growth and culture system that sustained growth
beyond 24hrs (Catrenich and Makin, 1991; Ho and Vijayakumari, 1993; Deshpande et al., 1995). In this study, slightly higher concentrations were achieved in a limited number of flasks when incubation was extended to 16 days. The most likely point of concentration occurred during repeated sampling on a daily basis or when flushing the flasks with fresh microaerophilic gas mixture after each sampling. Most studies with broths have incorporated antibiotics into the media to help minimize concentration (Ho and Vijayakumari, 1993; Deshpande et al., 1995). However, antibiotics were not added in this comparative experiment to prevent confounding by a possible interaction between the antibiotics and the media. This study validates a practical and efficient system for large numbers of *H. pylori* cells in liquid media without using cost-prohibitive continuous culture system and other testing, and it indicates that this medium can be used for *H. pylori* growth in further next testing.

The rate of growth and subsequent colony size increased in both media routinely used to culture *H. pylori*. BHIA showed continuous growth rate, measured with log10^7 CFU/ml in dilution range 10^1, 10^2 and 10^3 after on plate counts, however, the mean size of the colonies was larger on BHIA. Because *H. pylor* grows slowly in the laboratory, any improvement in colony size is an important consideration. *H. pylori* cultures are routinely incubated for 16 days before colonies are visible on BHI media, so the increased colony size observed on BHI broth is particularly noteworthy.

Results of kinetic growth study showed a normal growth culture i.e. HP-B_1, HP-B_2, HP-B_3 and HP-B_4. The bacteria were found to grow in the media normally and were taken for further studies.

5.3. *In vitro* analysis

*H. pylori* eradication regimen usually entails a proton pump inhibitor (Pantoprazole) in combination with one of the antimicrobial agents. However, various
clinical trials and in vitro studies have reported *H. pylori* antimicrobial resistance all over the world that represents a serious public health challenge. *H. pylori* antimicrobial resistance varies between different geographical regions. Therefore, health care awareness of the *H. pylori* susceptibility to each of the commonly used antibiotics is necessary to be able to recommend the most effective therapy regimen. Patient noncompliance and the location of the bacterium which is beneath the gastric epithelium and out of reach of antibiotics are among the most common causes of treatment failure (Duck *et al.* 2004; Tolia *et al.*, 2000).

The antibiotics used in common clinical practice to treat various infection, either alone or in combination are Clarithromycin, Amoxicillin, Tetracycline, Metronidazole, Cefotaxime, Ceftazidime, Ceftriaxone, Piperacillin, Tazobactum, Ciprofloxacin, Streptomycin, Erythromycin, Gentamycin, Tobramycin, Amikacin, Nitilmicin, and Vancomycin. The combination therapy of antibiotics with pantoprazole has been attempted in clinical practices.

Analysis of susceptibility of combination by cup plate, MIC, time kill curve, and resistance developments against *H. pylori in vitro* has not been evaluated so far (Goodwin *et al.*, 1986). In the present study, the activity of most of the above mentioned antibiotics against *H. pylori* exhibited a bimodal phenomenon, suggesting that clinical and ATCC groups of *H. pylori* are either highly susceptible or moderately susceptible to the combination. However, there are no generally accepted criteria for the inhibitory zone size that differentiates susceptible and resistant developments in the combination drugs by these techniques, although several studies have used this technique (Boero *et al.*, 1991; DeCross, *et al.*, 1993; Gasperoni *et al.*, 1991; Goodwin *et al.*, 1986; Goodwin *et al.*, 1988; Hirschli *et al.*, 1993; Knapp *et al.*, 1991; Mathai *et al.*, 1990; Pavicic *et al.*, 1993). The cup plate method was the found to be the method of
choice for susceptibility testing on all strains of \textit{H. pylori} groups for combination and alone drugs. The microdilution broth method was used for growing \textit{H. pylori} in liquid media and the potential for contamination (DeCross \textit{et al.}, 1993; Goodwin \textit{et al.}, 1986).

5.3.1. \textit{In vitro} analysis of Pantoprazole-Clarithromycin (PC), a fixed dose combination and alone drugs

It was reported that Pantoprazole was shown to be the superior complementary treatment to endoscopic therapy in high-risk bleeding ulcers (Hsu \textit{et al.}, 2004; Huggins \textit{et al.}, 2003), as Pantoprazole appeared to be superior to the H\textsubscript{2} receptor antagonists (Huggins \textit{et al.}, 2003). One of the major advantages of Pantoprazole is that no change in dosage is necessary when switching from (intravenous) IV to oral therapy or vice versa (Pisegna, 2001). Pantoprazole therapy combined with anti-infective agents like Tetracycline, can effectively eradicate \textit{H. pylori} gastric infection. It has also been used in those patients with pathological hyper secretion associated with Zollinger-Ellison syndrome (Metz \textit{et al.}, 2001). The Pantoprazole also has low therapeutic potential and bacteriocidal activity at neutral pH, there by requiring short incubation periods against \textit{H. pylori} (Diez Enciso, 1999).

\textit{H. pylori} resistance rate to clarithromycin has been described as 2-50\% in various studies (Malaty \textit{et al.}, 1996). Macrolids are commonly prescribed in pediatric age group for otitis media and upper airway infections. Since a cross over resistance mechanism among different types of macrolids develops rapidly, the \textit{H. pylori} resistance rate is correlated to national level of macrolid consumption. Moreover according to certain studies, resistance to clarithromycin is more frequently seen in children compared with adults (Duck \textit{et al.}, 2004).

Clarithromycin is acid stable, readily absorbed, gets diffused into most tissues, gets phagocyted and actively transported to the site of infection. Clarithromycin prevents
bacteria from growing by interfering with their protein synthesis. It binds to the subunit 50S of the bacterial ribosome and thus inhibits the translation of peptides (Graham, 1993). This antibiotic is currently accepted as the most effective drug in *H. pylori* infection, with a 42% eradication rate (Graham, 1995; Hunt, 1996). Moreover, the eradication rate increased with the use of antibiotics Clarithromycin and combinations including PPIs Pantoprazole (Daw *et al.*, 1991; Graham, 1993; Iwahi *et al.*, 1991).

The *in vitro* study, low eradication rates, Proton Pump Inhibitor- based effective drug of clarithromycin in two regimens (Pantoprazole plus clarithromycin) have been reported in *H. pylori* infection (Lamouliatte *et al.*, 2000; Rinaldi *et al.*, 1999). The susceptibility data from our study demonstrated that Pantoprazole-Clarithromycin (PC) combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC value than Clarithromycin and Pantoprazole alone, suggesting higher bactericidal activity and more efficacy in PC in HP-B₁, HP-B₂, HP-B₃ and HP-B₄ of *H. pylori* groups. This was confirmed by time-kill analysis, which demonstrated that PC has better bactericidal activity than Clarithromycin and Pantoprazole, even at a concentration of 2 X the MIC after 12 hours. Indeed, in all organisms under study, Pantoprazole-Clarithromycin, demonstrated similar pattern of bactericidal activities when compared with alone.

The resistance developments of our study demonstrated that the Pantoprazole-Clarithromycin combination has higher zone value were found in well concentration 30μg, 20μg, and 10μg as well as higher microbial efficacy as compared to Clarithromycin alone in all *H. pylori* groups. The study suggested that there is less chance of generation of resistance as the 10μg concentration of this combination is also having bactericidal effect.
5.3.2. *In vitro* analysis of Pantoprazole-Amoxicillin (PA), a fixed dose combination and alone drugs

Amoxicillin is an penicillin group antibiotic usually susceptible to the *H. pylori* infection particular gram negative infection (Dore *et al.*, 1999). Treatment usually involves a combination of antibiotic and acid suppressors proton pump inhibitor protection. (Noophun *et al.*, 2004). Very high resistance rates to amoxicillin have been reported in some prevalence studies (Godoy *et al.*, 2003 and Wu *et al.*, 2000).

The reliable treatments of *H. pylori* infection has been difficult and successful regimens generally require two or more antimicrobial drugs coupled with an acid inhibitor (Graham *et al.*, 1996 and Megraud, 1997). Results with the dual therapy that combined pantoprazole with amoxicillin have varied widely (Penston, 1997 and Van der Hulst *et al.*, 1996).

The *in vitro* study, low eradication rates, PPI- based effective drug of Amoxicillin in two regimen (Pantoprazole and Amoxicillin) have been reported in *H. pylori* infection (Dore *et al.*, 1999 and Graham *et al.*, 1996). The susceptibility data from our study demonstrated that PA combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC value than Amoxicillin and Pantoprazole alone, suggesting higher bactericidal activity in PA in isolated gastric biopsy (HP-B₁, HP-B₂, HP-B₃) and ATCC 43504 (HP-B₄) of *H. pylori* strains. This was confirmed by time-kill analysis, which demonstrated that PA has better bactericidal activity than Amoxicillin and Pantoprazole alone, even at a concentration of 2X the MIC after 12 hours. Indeed, in all organisms under study, PA, demonstrated similar pattern of bactericidal activities when compared with alone.

The resistance developments of our study demonstrated that the PA combination has higher zone value were found in well concentration 30μg, 20μg, and 10μg as well
as higher microbial efficacy as compared to Amoxycillin alone in all *H. pylori* groups.

The study suggested that there is less chance of generation of resistance as the 10µg concentration of this combination is also bactericidal in nature. There is less chance of generation of resistance to either of the combination.

5.3.3. *In vitro* analysis of Pantoprazole-Tetracycline (PT), a fixed dose combination and alone drugs

Tetracycline is acid stable, readily absorbed, diffuses into most tissues, phagocytes and is actively transported to the site of infection. Tetracycline prevents bacteria growth by interfering with their protein synthesis. It binds to the subunit 30S of the bacterial ribosome and thus inhibits the translation of peptides (Piolett *et al.*, 2001). These Antibiotics is currently accepted as the most effective drug in *H. pylori* infection, with a 76% eradication rate (Triebel *et al.*, 2002). Moreover, the eradication rate increased when combined with other antibiotics (Kim *et al.*, 2001; Wu *et al.*, 2000), including combinations of PPIs - Pantoprazole (Wu *et al.*, 2000).

The in vitro study, low eradication rates, PPI- based effective drug of Tetracycline in two regimens (Pantoprazole plus Tetracycline) have been reported in *H. pylori infection* (Wu *et al.*, 2000; Graham *et al.*, 1993). The susceptibility data from our study demonstrated that PT, in a fixed dose combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC values than Tetracycline or Pantoprazole, suggesting higher bactericidal activity for PT in isolated gastric biopsy *H. pylori* cultured groups (HP-B1, HP-B2, HP-B3) and ATCC 43504 (HP-B4). These findings were supported and confirmed by time-kill analysis, which demonstrated that PT has better bactericidal activity than Tetracycline or Pantoprazole, even at a concentration of 2 X the MIC after 12 hours. These superior bactericidal activities of PT compared to single treatment showed a similar pattern in all the
organisms studied.

The resistance developments of our study demonstrated that the PA combination has higher zone value were found in well concentration 30μg, 20μg, and 10μg as well as higher microbial efficacy as compared to Tetracycline alone in all H. pylori groups. The chance of resistance development is least in this combination, as this is the most active combination in vitro studies.

5.3.4. In vitro analysis of Pantoprazole- Metronidazole (PM), a fixed dose combination and alone drugs

Metronidazole is one of the most successful drugs used in combination to eradicate H. pylori. However, distribution of its MIC against H. pylori in vitro has not been regularly shown (Huaxiang et al., 1994). It is generally accepted that metronidazole MICs of more than 2mg/l are resistant for H. pylori strains (Glupczynski et al., 1990). These Antibiotics is currently accepted as the most effective drug in H. pylori infection, with a 80.2 % eradication rate (Xia et al., 1993). Moreover, the eradication rate increased with the use of antibiotics metronidazole and combinations including PPIs pantoprazole.

Metronidazole resistance is strongly believed to be caused by H. pylori genetic drift and adaptation to environment (Tolia et al., 2000; Sisson et al., 2000). Increased prescription of metronodazole for parasitic infection among children and infection with a resistant strain H. pylori are the other factors leading to high metronidazole resistance rate (Kim et al., 2001).

The in vitro study, low eradication rates, PPI- based effective drug of metronidazole in two regimen (Pantoprazole plus Metronidazole) have been reported in H. pylori infection (Goodwin et al., 1986). Metronidazole is a compound that has potent bacterial activities against gram negative organism H. pylori, particularly
Clinically relevant pathogen (Graham et al., 1995). The susceptibility data from our study demonstrated that PM, a fixed dose combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC value than Metronidazole and Pantoprazole, suggesting higher bactericidal activity in PM in isolated gastric biopsy and ATCC 43504 H. pylori groups (HP-B₁, HP-B₂, HP-B₃, and HP-B₄). This was confirmed by time-kill analysis, which demonstrated that PM has better bactericidal activity than Metronidazole and Pantoprazole, even at a concentration of 2X the MIC after 12 hours. Indeed, in all organisms under study, PM, demonstrated similar pattern of bactericidal activities when compared with alone.

The resistance developments of our study demonstrated that the PM combination to have higher zone value and higher microbial efficacy were found at well concentration 30μg, 20μg, and 10μg as compared to Metronidazole alone in all H. pylori groups.

5.3.5. In vitro analysis of Pantoprazole-Cefotaxime, a fixed dose combination and alone drugs

Cefotaxime is a novel parenteral cephalosporin with activity against gram-negative organisms (Barza et al., 1976; Bennett et al., 1966). These Antibiotics is currently accepted as the effective drug in H. pylori infection, with a 80.2 % eradication rate (Fong et al., 1976; Barza et al., 1976). The in vitro study, low eradication rates, PPI-based effective drug of Cefotaxime in two regimen (Pantoprazole plus Cefotaxime) have been reported in H. pylori infection. Cefotaxime is a compound that has potent bacterial activities against gram negative organism H. pylori, particularly clinically relevant pathogen (NAEJA Pharmaceutical Inc. Canada). The susceptibility data from our study demonstrated that Pantoprazole-Cefotaxime, a fixed dose combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and
lower MIC value than Cefotaxime and Pantoprazole, suggesting higher bactericidal activity in Pantoprazole-Cefotaxime combination in isolated gastric biopsy and ATCC 43504 H. pylori groups (HP-B₁, HP-B₂, HP-B₃, and HP-B₄). This was confirmed by time-kill analysis, which demonstrated that Pantoprazole-Cefotaxime, a fixed dose combination has better bactericidal activity than Cefotaxime and Pantoprazole, even at a concentration of 2 X the MIC after 12 hours. Indeed, in all organisms under study, Pantoprazole-Cefotaxime, demonstrated similar pattern of bactericidal activities when compared with alone.

The resistance developments of our study demonstrated that the Pantoprazole-Cefotaxime, a fixed dose combination has higher zone value were found in well concentration 30 μg, 20 μg, and 10 μg as well as higher microbial efficacy as compared to Cefotaxime alone in all H. pylori groups.

5.3.6. *In vitro* analysis of Pantoprazole-Ceftazidime, a fixed dose combination and alone drugs

Ceftazidime is a broad spectrum, third generation parenteral cephalosporin effective against many Gram negative organisms. Serious infections of the respiratory tract and gastrointestinal tract infections (Ayrton, 1981; Barza, 1982).

The pharmacokinetic properties of Ceftazidime, a third generation cephalosporin, and MICs of Ceftazidime for some Gram-negative strains isolated from clinical cases were determined. An efficacy predictor, measured as the time over which the active drug exceeds the bacteria MICs was calculated. Serum Ceftazidime disposition was best fitted by a bi-compartmental and a mono-compartmental open model with first-order elimination after IV and IM dosing, respectively (Cars and Qgren, 1985; Dawson, 1981). Ceftazidime is a compound that has potent bacterial activities against gram negative organism *H. pylori*, particularly clinically relevant pathogen (NAEJA
Pharmaceutical Inc. Canada). The susceptibility data from our study demonstrated that Pantoprazole-Ceftazidime, a fixed dose combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC value than Ceftazidime and Pantoprazole, suggesting higher bactericidal activity in Pantoprazole-Ceftazidime combination in isolated gastric biopsy and ATCC 43504 H. pylori groups (HP-B₁, HP-B₂, HP-B₃, and HP-B₄). This was confirmed by time-kill analysis, which demonstrated that Pantoprazole-Ceftazidime, a fixed dose combination has better bactericidal activity than Ceftazidime and Pantoprazole, even at a concentration of 2X the MIC after 12 hours. Indeed, in all organisms under study, Pantoprazole-Ceftazidime, demonstrated similar pattern of bactericidal activities when compared with alone.

The resistance developments of our study demonstrated that the Pantoprazole-Ceftazidime, a fixed dose combination has higher zone value were found in well concentration 30μg, 20μg, and 10μg as well as higher microbial efficacy as compared to Ceftazidime alone in all H. pylori groups.

5.3.7. In vitro analysis of Pantoprazole-Ceftriaxone, a fixed dose combination and alone drugs

Ceftriaxone is a third generation cephalosporin which has a broad spectrum of activity in vitro (Allaz et al., 1979). Its activity against most gram-negative bacteria was greater than those of some of the other new broad-spectrum cephalosporins. The in vitro activity of ceftriaxone against gram-negative anaerobic organisms compares favorably with those of other cephalosporins (Angehrn et al., 1980). Ceftriaxone inhibits the synthesis in the bacterial cell wall. Like other cephalosporins of the 'third generation', it has a very broad anti-bacterial spectrum and it is stable against most β-lactamases. Ceftriaxone owes its popularity mainly to its relatively long plasma half-
life. (Craig, 1980).

Ceftriaxone is a compound that has potent bacterial activities against gram negative organism *H. pylori*, particularly clinically relevant pathogen (NAEJA Pharmaceutical Inc. Canada). The susceptibility data from our study demonstrated that Pantoprazole-Ceftriaxone, a fixed dose combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC value than Ceftriaxone and Pantoprazole, suggesting higher bactericidal activity in Pantoprazole-Ceftriaxone combination in isolated gastric biopsy and ATCC 43504 *H. pylori* groups (HP-B1, HP-B2, HP-B3, and HP-B4). This was confirmed by time-kill analysis, which demonstrated that Pantoprazole-Ceftriaxone, a fixed dose combination has better bactericidal activity than Ceftriaxone and Pantoprazole, even at a concentration of 2 X the MIC after 12 hours. Indeed, in all organisms under study, Pantoprazole-Ceftriaxone, demonstrated similar pattern of bactericidal activities when compared with alone.

The resistance developments of our study demonstrated that the Pantoprazole-Ceftriaxone, a fixed dose combination has higher zone value were found in well concentration 30μg, 20μg, and 10μg as well as higher microbial efficacy as compared to Ceftriaxone alone in all *H. pylori* groups.

5.3.8. *In vitro* analysis of Pantoprazole-Ciprofloxacain, a fixed dose combination and alone drugs

Ciprofloxacain is one of a second generation of fluorinated quinolones structurally related to nalidixic acid. The primary mechanism of action of ciprofloxacain is inhibition of bacterial DNA gyrase. It is a broad spectrum antibacterial drug to which most Gram-negative bacteria are highly susceptible *in vitro*. It attains concentrations in most tissues and body fluids which are at least equivalent to the MICs designated as the breakpoint

Ciprofloxacin generally appeared to be at least as effective as alternative orally administered antibacterial drugs in the indications in which they were compared, and in some indications, to parenterally administered antibacterial therapy. The drug is also well tolerated. Thus, as an orally active, broad spectrum and potent antibacterial drug, Ciprofloxacin offers a valuable alternative to broad spectrum parenterally administered antibacterial drugs for use in a wide range of clinical infections (Egerbacher *et al.*, 2000, Curtis *et al.*, 1990). Ciprofloxacin is a compound that has potent bacterial activities against gram negative organism *H. pylori*, particularly clinically relevant pathogen (Boyanova *et al.*, 2000; NAEJA Pharmaceutical Inc. Canada). The susceptibility data from our study demonstrated that Pantoprazole-Ciprofloxacin, a fixed dose combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC value than Ciprofloxacin and Pantoprazole, suggesting higher bactericidal activity in Pantoprazole- Ciprofloxacin combination in isolated gastric biopsy and ATCC 43504 *H. pylori* groups (HP-B₁, HP-B₂, HP-B₃, and HP-B₄). This was confirmed by time-kill analysis, which demonstrated that Pantoprazole-Ciprofloxacin, a fixed dose combination has better bactericidal activity than Ciprofloxacin and Pantoprazole, even at a concentration of 2X the MIC after 12 hours. Indeed, in all organisms under study, Pantoprazole-Ciprofloxacin, demonstrated similar pattern of bactericidal activities when compared with alone.

The resistance developments of our study demonstrated that the Pantoprazole- Ciprofloxacin, a fixed dose combination has higher zone value were found in well concentration 30μg, 20μg, and 10μg as well as higher microbial efficacy as compared to Ciprofloxacin alone in all *H. pylori* groups.
5.3.9. *In vitro* analysis of Pantoprazole-Erythromycin, a fixed dose combination and alone drugs

Erythromycin can be considered the prototype of macrolide antibiotics. These drugs inhibit the ribosomal protein synthesis in bacteria and thus have a bacteriostatic and bactericidal effect. Erythromycin has a similar action spectrum as penicillin and includes in particular clarithromycin (Carson *et al.*, 1993; Seppala *et al.*, 1992, Curtis *et al.*, 1990).

Erythromycin is a compound that has potent bacterial activities against gram negative organism *H. pylori*, particularly clinically relevant pathogen (Isabel Garcia-Arata *et al.*, 1999; NAEJA Pharmaceutical Inc. Canada). The susceptibility data from our study demonstrated that Pantoprazole-Erythromycin, a fixed dose combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC value than Erythromycin and Pantoprazole, suggesting higher bactericidal activity in Pantoprazole-Erythromycin combination in isolated gastric biopsy and ATCC 43504 *H. pylori* groups (HP-B₁, HP-B₂, HP-B₃, and HP-B₄). This was confirmed by time-kill analysis, which demonstrated that Pantoprazole-Erythromycin, a fixed dose combination has better bactericidal activity than Erythromycin and Pantoprazole, even at a concentration of 2 X the MIC after 12 hours. Indeed, in all organisms under study, Pantoprazole-Erythromycin, demonstrated similar pattern of bactericidal activities when compared with alone.

The resistance developments of our study demonstrated that the Pantoprazole-Erythromycin, a fixed dose combination has higher zone value were found in well concentration 30μg, 20μg, and 10μg as well as higher microbial efficacy as compared to Erythromycin alone in all *H. pylori* groups.
5.3.10. *In vitro* analysis of Pantoprazole-Gentamicin, a fixed dose combination and alone drugs

Gentamicin is a broad spectrum aminoglycoside antibiotic. Aminoglycosides work by binding to the bacterial 30S ribosomal subunit, causing misreading of t-RNA, leaving the bacterium unable to synthesize proteins vital to its growth (Kaye *et al.*, 1974; Riff *et al.*, 1971).

Aminoglycosides are useful primarily in infections involving anaerobic, Gram-negative bacteria. Aminoglycosides like Gentamicin "irreversibly" bind to specific 30S-subunit proteins and 16S rRNA. Specifically Gentamicin binds to four nucleotides of 16S rRNA and a single amino acid of protein S12. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids are inserted into the polypeptide leading to nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes (Jao and Jackson, 1964; Riff *et al.*, 1971). Gentamicin is a compound that has potent bacterial activities against gram negative organism *H. pylori*, particularly clinically relevant pathogen (NAEJA Pharmaceutical Inc. Canada). The susceptibility data from our study demonstrated that Pantoprazole-Gentamicin, a fixed dose combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC value than Gentamicin and Pantoprazole, suggesting higher bactericidal activity in Pantoprazole-Gentamicin combination in isolated gastric biopsy and ATCC 43504 *H. pylori* groups (HP-B1, HP-B2, HP-B3, and HP-B4). This was confirmed by time-kill analysis, which demonstrated that Pantoprazole-Gentamicin, a fixed dose combination has better bactericidal activity than Gentamicin and Pantoprazole, even at a concentration of 2 X the MIC after 12 hours. Indeed, in all organisms under study, Pantoprazole-Gentamicin, demonstrated similar pattern of bactericidal activities when compared with alone.
The resistance developments of our study demonstrated that the Pantoprazole-Gentamicin, a fixed dose combination has higher zone value were found in well concentration 30μg, 20μg, and 10μg as well as higher microbial efficacy as compared to Gentamicin alone in all H. pylori groups.

5.3.11. In vitro analysis of Pantoprazole-Tobramycin, a fixed dose combination and alone drugs

Tobramycin is an aminoglycosides group antibiotic. Tobramycin fights infections that are caused by anaerobic bacteria. Tobramycin is used to treat bacterial infections of the stomach. Tobramycin act by inhibiting synthesis of protein in bacterial cell. In vitro tests demonstrate that Tobramycin is bactericidal (NCCLS, 1993).

Tobramycin is a compound that have potent bacterial activities against gram negative organism H. pylori, particularly clinically relevant pathogen (Curtis et al., 1990). The susceptibility data from our study demonstrated that Pantoprazole-Tobramycin, a fixed dose combination have higher zone diameter (significant reduction, P value is < 0.0001 in all groups), and lower MIC value than Tobramycin and Pantoprazole, suggesting higher bactericidal activity in Pantoprazole-Tobramycin combination in isolated gastric biopsy and ATCC 43504 H. pylori groups (HP-B1, HP-B2, HP-B3, and HP-B4). This was confirmed by time-kill analysis, which demonstrated that Pantoprazole-Tobramycin, a fixed dose combination has better bactericidal activity than Tobramycin and Pantoprazole, even at a concentration of 2X the MIC after 12 hours. Indeed, in all organisms under study, Pantoprazole-Tobramycin demonstrated similar pattern of bactericidal activities when compared with alone.

The resistance developments of our study demonstrated that the Pantoprazole-Tobramycin, a fixed dose combination has higher zone value were found in well concentration 30μg, 20μg, and 10μg as well as higher microbial efficacy as compared
to Tobramycin alone in all *H. pylori* groups.

In summary, the results of the mean value of zone diameter, resistance developments, MIC and time kill studies are concordant for the three gastric biopsy (HP-B₁, HP-B₂, HP-B₃) and one ATCC 43504 (HP-B₄) of *H. pylori* strains. Pantoprazole-Antibiotics, a fixed dose combination has shown better bactericidal effect than Pantoprazole and Antibiotics alone in organisms under study.

5.3.12. *In vitro* efficacy of different combinations and alone

The susceptibility, MIC, and resistance developments data from our study demonstrates that Pantoprazole-Antibiotics (Piperacillin, Tazobactum, Streptomycin, Amikacin, Nitlimicin, and Vancomycin), a fixed dose combination and antibiotics alone were not having bacterial inhibition properties and bactericidal activity after *in vitro* analysis (not significant reduction), in isolated gastric biopsy and ATCC groups (HP-B₁, HP-B₂, HP-B₃ and HP-B₄).

In summary, the results of the zone diameter, resistance developments, MIC and time kill curve studies are not concordant for the three gastric biopsy (HP-B₁, HP-B₂, HP-B₃) and ATCC 43504 (HP-B₄) of *H. pylori* strains.

*In vitro* results showed that the combination of Pantoprazole-Tetracycline (A), Pantoprazole-Clarithromycin (B), and Pantoprazole-Amoxicillin (C) were found to be more effective with higher bacterial activity against *H. pylori*. These combinations were taken for further *in vivo* studies.

5.4. *In vivo* studies

5.4.1. *In vivo* studies for stomach

Ulceration is caused by an imbalance between damaging factors (gastric acid and peptic enzymes) and protective factors (gastric mucus secretion, local secretion of alkali). *H. pylori* appears to cause direct damage to the epithelial cells as well as
Discussion

secreting enzymes that break down the surface mucus, this allows access of acid to mucosal tissues leading to the formation of an acute ulcer. If the process proceeds unchecked, then the ulcer can erode through the full thickness of the stomach or duodenal wall, leading to perforation and escape of gut contents into the peritoneal cavity. Most commonly, however, the destructive process is arrested by an acute inflammatory response. Tissue repair then being with the formation of granulation tissue, repair may be effective if conditions are favourable, leaving a fibrous scar. If tissue destruction continues, the concurrent organisation and repair result in chronic inflammation. A chronic peptic ulcer reflects a dynamic balance between tissue destruction and tissue repair (Parasad et al., 1994).

*H. Pylori* peptic ulcers are treated with drugs that kill the bacteria, reduce stomach acid, and protect the stomach lining. Antibiotics are used to kill the bacteria. Pantopazole for acid-suppressing drugs might be used. Various groups of animals, as specified in materials and methods were taken for studies. Therefore, *in vivo* therapeutic efficacies of the selected combination were checked in mouse model.

5.4.1.1. *In vivo* Study of Pantoprazole-Tetracycline (A), a fixed dose combination drugs

In case of ‘A’ the stomach infection was clearly marked. After treatment for 15 days with Pantoprazole and Tetracycline, a fixed dose combination (A), tissue degradation and *H. pylori* infection (H) was evident. Necrotic tissue (NE), Submucosa (u), muscularis mucosae (s), and neutrophils (N) were also marked.

After treatment for 30 days with Pantoprazole and Tetracycline (A) still situs of *H. pylori* infection (H) was seen. Mucosa (m), muscularis mucosae and Submucosa also found with degradation. The treatment of 45 days started showing recovery of the histological attributes with regeneration of mucosa (R).
There has been gradual recovery in treatment of infection with Pantoprazole-Tetracycline (PT), a fixed dose combination after 60 days of treatment there was completely recovery.

5.4.1.2. *In vivo* Study of Pantoprazole-Clarithromycin (B), a fixed dose combination drugs

In case of ‘B’ the stomach infection was clearly marked. After treatment for 15 days with Pantoprazole combination with Clarithromycin (B), tissue degradation and *H. pylori* infection (H) was evident. Necrotic tissue (NE) and muscularis mucosae (s) were also marked.

After treatment for 30 days with Pantoprazole and Clarithromycin (B) still situs of *H. pylori* infection (H) was not seen. Necrotic tissue was also marked. The treatment of 45 and 60 days started showing recovery of the histological attributes with regeneration of mucosa (R). There has been gradual recovery in treatment of infection with Pantoprazole-Clarithromycin (PC), a fixed dose combination after 60 days of treatment there was completely recovery.

5.4.1.3. *In vivo* Study of Pantoprazole-Amoxicillin (C), a fixed dose combination drugs

In case of ‘C’ the stomach infection was clearly marked. After treatment for 15 days with Pantoprazole combination with Amoxicillin (C), tissue degradation and *H. pylori* infection (H) was evident. Necrotic tissue (NE) and mucosa (m) were also marked.

After 30 days of treatment with Pantoprazole and Amoxicillin (C) still sites of *H. pylori* infection (H) were not observed in degenerated mucosa, muscularis mucosae and submucosa were also marked. The treatment of 45 and 60 days showed recovery of histological attributes with regeneration of mucosa and muscularis mucosae.
Gradual recovery of infection was observed on treatment with Pantoprazole-Amoxicillin (C), a fixed dose combination and complete recovery was observed after 60 days of treatment.

5.4.2. In vivo studies for Intestine

An ulcer is a sore or raw area, in the lining of some part of the intestinal tract. Peptic ulcers typically occur in either the stomach or in the duodenum, which is the first several inches of the small intestine just below the stomach (Fischbach et al., 1997).

5.4.2.1. In vivo Study of Pantoprazole-Antibiotics (‘A’, ‘B’ and ‘C’), a fixed dose combination drugs

In case of small intestine there was minor infection reported after the induction of infection in all groups ‘A’, ‘B’ and ‘C’. After treatment with Pantoprazole and Tetracycline (‘A’), a fixed dose combination for 15 days almost normal histological structure was marked with little degradation and H. pylori infection (i) at Lamina propria (p), but after 15 days treatment with a fixed dose combination of ‘B’ and ‘C’ almost normal histological structure was observed with little degradation at Lamina propria and little degradation at Crypt of lieberkuhn (c).

Normal histological structure of small intestine was seen at 30, 45, and 60 days of treatment with Pantoprazole and Antibiotic, a fixed dose combination. Normal villi, Lamina propria, Crypt of lieberkuhn and muscularis mucosae were observed. There was marked improvement in 15 days of treatments and in all groups the infection was recovered even after 30 days of therapy.

5.4.3. In vivo studies for Rectum

It is a chronic, uncommon, inflammatory, benign disorder of young adults, influencing the rectum, where it is frequently linked to painful or abnormal defecation with bleeding, passage of mucus, straining during defecation, and a sense of incomplete
evacuation which is related to mucosal prolapse and rarely to underlying malignancy (Eigenmann et al., 1992). Rectal ulcer syndrome (SRUS) is an under-diagnosed disease with very low disease awareness and with a varied clinical presentation, protean endoscopic appearance, yet characteristic histological features in all over the world. It is not a very common disease. It can be defined as the ulceration of the rectal wall. Together with this ulceration, histological factors also play an important role. People who have chronic spasm or injuries of the rectum constitute the disease population of rectal ulcer syndrome (For et al., 1983; Kumar et al., 1994).

5.4.3.1. *In vivo* Study of Pantoprazole-Antibiotics (‘A’, ‘B’ and ‘C’), a fixed dose combination drugs

In case of Rectum in all groups (‘A’, ‘B’ and ‘C’) showed normal histopathological structure. Control rectum showed normal structure of crypt of lieberkuhn (C), muscularis mucosae (M), lamina propria (N) and Submucosa (E).

After infection and in all groups of treatments of 15, 30, 45 and 60 days with Pantoprazole and Antibiotics (‘A’, ‘B’ and ‘C’), showed almost normal structure lamina propria (N), crypt of lieberkuhn (C), muscularis mucosae (M), vain (A), and Submucosa (E) were marked as normal. There was no difference in histological structure after treatment and in untreated rectum.

In all groups of treatments, best *in vivo* results was found in the case of combination of Pantoprazole-Tetracycline, as compared with Pantoprazole-Clarithromycin and Pantoprazole-Amoxicillin. The histological recovery in case of stomach ulcer was almost similar in all combination of antibiotics with Pantoprazole. In time kill curve analysis, there has been maximum reduction in the culture of *H. pylori* growth in the case of Pantoprazole-Tetracycline followed by Pantoprazole-Clarithromycin and Pantoprazole-Amoxicillin.
This study clearly indicates that the combination of Pantoprazole-Tetracycline, Pantoprazole-Clarithromycin and Pantoprazole-Amoxicillin are effective in treatment of infections caused by *H. pylori* in laboratory condition. The *in vitro* results showed Pantoprazole-Tetracycline to be most effective for eradication of *H. pylori*. Pantoprazole mediated successful treatments of *H. pylori* infections were done in laboratory animals in combination with Tetracycline, Clarithromycin and Amoxicillin. Highest efficacy was reported of Pantoprazole-Tetracycline combination. It may be concluded that any of these three combinations can be used for the treatment of *H. pylori* infections in humans after successful clinical evaluation.