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Anti-*Helicobacter pylori* activity and inhibition of *Helicobacter pylori*-induced release of IL-8 in AGS cells by plant extracts

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Plants used in popular diet were studied for anti-*Helicobacter pylori* activity and their effect on the expression of interleukin-8 (IL-8) from *H. pylori* infected gastric epithelial cells. Extracts were prepared of *Allium sativum (A. sativum)*, *Cuminum cyminum (C. cyminum)*, *Piper nigrum* (*P. nigrum*) and their mix in two different dilutions. AGS cell line and *H. pylori* strains were used for co-culture experiments. Extracts bactericidal activity was determined by a viable colony count. ELISA (Enzyme linked immunosorbent assay) was used to determine IL-8 expression. DNA extracted from bacterial cells was used for polymerase chain reaction (PCR) of cytotoxin-associated gene (*cagA*) and *E (cagE)*. Results revealed *H. pylori* strains sensitivity to *A. sativum* (5.5 mg/ml) was 57% (39/69) (p=0.06), and to 11 mg/ml was 65% (45/69) (p=0.02) compared to amoxicillin, respectively. *CagE* positive *H. pylori*, 37% (11/30) (p=0.02) were sensitive to plant mixture (23.5 mg/ml), 60% (18/30) (p<0.001) to plant mix (47 mg/ml), respectively. The expression of IL-8 by AGS cells treated with plant mix, (P=0.0037), *C. cyminum* (P<0.0001), *P. nigrum* (P=0.0046) and *A. sativum* (P=0.0021), respectively compared to positive and negative controls. Thus, dietary plants demonstrated an anti-*H. pylori* effect. They reduced IL-8 expression from the *H. pylori* infected AGS cells.

Key words: *Helicobacter pylori; Allium sativum*, *Cuminum cyminum*, *Piper nigrum,* gastric epithelial cells, interleukin-8, ELISA, *cagA*, *cagE.*

INTRODUCTION

The gram-negative bacterium *Helicobacter pylori* colonize the stomach where it is associated with chronic gastritis, gastric and duodenal ulcer and mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma (Blaser et al., 1995; Cover and Blaser, 1999). The current commonly prescribed regime for the eradication of *H. pylori* infection includes a triple therapy, which combines the antibiotic clarithromycin (CLR) and amoxicillin (AMX) with a proton pump inhibitor such as omeprazole. This regimen is associated with side-effects and fails to eliminate infection in 30% of patients (Yakoob et al., 2010). The occurrence of strains resistant to CLR is expected to increase, and it is important to search for non-antibiotic substances with anti-*H. pylori* activity. In

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human gastric epithelial cells, *H. pylori* induce expression of genes associated with inflammation including activation of the nuclear factor kappa B (NF-κB), and production of interleukin (IL)-8 (Naumann, 2001). Among the cytokines induced in the gastric mucosa colonized by *H. pylori*, IL-8 is one of the major proinflammatory cytokines that plays a crucial role in the initiation and maintenance of inflammatory response (Kido et al., 2001). It is identified to function as a proangiogenic or carcinogenic factor based (Kido et al., 2001). Clinical diseases that follow *H. pylori* infection are shown to be associated with expression of the *cagA* gene, a part of cytotoxin-associated gene-pathogenicity island (cag-PAI). *CagE* is involved in the induction of pro-inflammatory cytokine interleukin-8 expression in gastric epithelial cells (Tummuru et al., 1995).

Plants are the source of natural compounds called phytochemicals that possess antimicrobial and anti-adhesive properties (Lampe, 1999). Medicinal plants have been used as traditional remedies in treating gastrointestinal diseases and their anti-*H. pylori* activity has been widely demonstrated *in vitro* (Stamatis et al., 2003; Ustun et al., 2006; Ndip et al., 2007; Shih et al., 2007). *A. sativum* (garlic) contains thiosulfinates which are responsible for the antibacterial activity (Jonkers et al., 1999; Iimuro et al., 2002; Sivam et al., 1997). *Cuminum cyminum* is a popular spice and its extract has been shown to be efficient at killing *H. pylori* (O'Mahony et al., 2005). Antibacterial activity of *Piper nigrum* has been demonstrated against penicillin G resistant strain of *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis* (Pundir and Jain, 2010; Perez and Anesini, 1994). The combination of *A. sativum*, *C. cyminum* and *P. nigrum* with mint are commonly used locally as a remedy for treatment of abdominal pain and vomiting. In a recent study, the seropositivity of *H. pylori* in children aged 11 to 15 years was described at 53.5% (Jafri et al., 2010). There is a high prevalence of *H. pylori* in Pakistani population however, the occurrence of duodenal ulcer (DU) and gastric carcinoma (GC) is much less. In this study we investigated the role of several constituent of the local cuisine which might be modulating the pathogenicity of the *H. pylori.* In view of the culinary significance of *A. sativum*, *P. nigrum* and *C. cyminum* and their medicinal use in gastrointestinal motility disorders, we studied the effects of the individual extracts and their combination in two different dilutions against *H. pylori*. We determined the effectiveness of *A. sativum*, *P. nigrum* and *C. cyminum* and their mixture as anti-*H. pylori* agents and determined whether their extracts can block the expression of IL-8 from AGS epithelial cell by *H. pylori* clinical isolates.

METHODS AND MATERIALS

Bacterial and cell culture

H. pylori strains were isolated from gastric antrum biopsies obtained from 60(80%) patients with nonulcer dyspepsia with gastritis and 15(20%) with duodenal ulcer. The mean age of the patients was 41±16 years (age range 26 to 70) and male to female ratio was 40:35. Seventy-five *H. pylori* strains sensitivity was tested to CLR, AMX, *A. sativum* (garlic), *P. nigrum*, C. *cyminum* and their mix. Fifty-eight (77%) of *H. pylori* isolates were collected for DNA extraction and determination of cag-PAI genes such as *cagA* and *cagE* gene. Some of the *cagA* gene amplified PCR products were sequenced and submitted to gene bank with sequence number assigned 1419039, 1419030 and 1419026.

AGS cells (ATCC CRL 1739; human gastric adenocarcinoma epithelial cell line) were cultured in RPMI (Sigma) supplemented with 10% de-complement fetal bovine serum (Invitrogen). Penicillin and streptomycin (GIBCO BRL) were also added if needed. In the *H. pylori* induced IL-8 secretion, the cell culture medium was not supplemented with antibiotics.

Plant material and preparation of crude extracts

The dried fruits of *P. nigrum* and *C. cyminum* as well as fresh *A. sativum* were bought from a local market (Juna Market) in Karachi, Pakistan. A specimen of each was preserved at the Herbarium of the Natural Product Research Division of the Department of Medicine, Aga Khan University, Karachi with the voucher number PN-JM-061-6-10; AS-JM-062-06-10 and CC-JM-063-06-10, respectively. For the extraction of the plant extract, a previously described method was followed with some modification (Williamson et al., 1998). They were weighed, grounded and soaked in 70% methanol for three days and filtered through muslin cloth and Whatman (Maidstone, UK) filter paper No. 1, simultaneously. This procedure was repeated three times, and all the filtrate was pooled and evaporated on a rotary evaporator (Model RE-111, Buchi, Flawil, Switzerland) under reduced pressure (-760 mm Hg) to get the final extract. The yield of the thick pasty mass for each was 6 to 8% (wt/wt). Dilutions of the crude extracts were prepared in distilled water/saline for experimentation. Extracts of *A. sativum*, *C. cyminum* and *P. nigrum* were mixed in equal quantity and then their dilutions were made in two different concentrations. Infusions were neutralized to pH 7.0. Extracts were stored in the dark at -20°C until use. Infusion of dried spices was used except for garlic which was used fresh.

Preparing different concentrations of the herbal extracts

Plant extracts were used in two different concentrations, *A. sativum* 5.5 and 11 mg/ml, *P. nigrum* 10 and 20 mg/ml, *C. cyminum* 8 and 16 mg/ml, respectively. Plant extract mix was prepared by mixing the three extracts in equal quantity and then using two different concentrations as 23.5 and 47 mg/ml. The tested doses of herbs correlated with their concentrations used in daily cuisine intake of these plants.

Susceptibility testing

Bacteria were grown for 5 days on blood agar plates supplemented with 5% sheep blood (Oxoid, UK) at 37°C under microaerobic conditions using anaerobic jars and Campygen strips (Oxoid, UK). Bactericidal activity of the herb extracts was determined by a viable colony count.

Clarithromycin and AMX were both used as control for determining *H. pylori* sensitivity against herbs extracts. One hundred microliters of a suspension of 10^8 bacteria/µl (McFarland No 3) was streaked on the blood agar plate (Oxoid, UK) with 5% defibrinated sheep blood. The control consisted of *H. pylori* incubated without antibiotic/plant extract for 4 days. Antimicrobial

Table 1. Sequences of oligonucleotide primers.

Primer	Primer sequence	Amplicon size (bp)	Location
CagA			
D008	GGTCAAAATGCGGTCATGG	297	1 cycle of 94°C for 5 min, 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 90 s, 1 cycle of 72°C for 5 min.
R008	TTAGAATAATCAACAAACATCACGCCAT		
cagE-F1	5'-GCGATTGTTATTGTGCTTGTAG-3'	329	
cagE-R1	5'-GAAGTGGTTAAAAAATCAATGCCCC-3'		

sensitivity of *H. pylori* isolates were detected by agar disk-diffusion procedure (Xia et al., 1996; Glupczynski, 1996). The antibiotic disks of 6 mm diameter (Oxoid, UK) AMX 10 µg/disk and CLR 15 µg/disk were placed on the plates and incubated at 37°C in a microaerophilic jar for 4 days and examined for the diameter of the inhibition zone, which was measured in millimeters. Isolates with diameters of inhibitory zones of < 30 mm were defined as resistant to CLR and < 18 mm to AMX while those with greater values as susceptible. The procedure was repeated for cultures that were defined as resistant. Bacterial strains were washed off the plates with and without exposure to plant extracts and bacterial pellets were stored at -20°C until used.

Extraction of genomic DNA

The bacterial cells on chocolate agar plate was washed twice with phosphate buffer saline (PBS, pH 8.0) then centrifuged at 3000 rpm for 20 min. *H. pylori* DNA was extracted by a phenol/chloroform method similar to the method previously described (Yakoob et al., 2000). Bacterial pellet was suspended in Tris-Cl buffer containing ethylenediaminetetraacetate (T.E pH 8.0) and lysozyme and was then incubated at 37°C for 30 min. The suspension was treated with sodium dodecyl sulphate, proteinase K and RNase A. DNA was extracted with phenol/chloroform/isoamyl alcohol, precipitated by sodium acetate and ice-cold absolute alcohol, and washed with ice cold alcohol (70%). The pellet of DNA was finally resuspended in TE buffer. DNA content and purity was determined by measuring the absorbance at 260 and 280 nm using a spectrophotometer (Beckman DU-600, USA).

***Cag* PAI PCR analysis**

PCR analyses were carried out with oligonucleotide pairs (Table 1) described previously (Covacci and Rappuoli, 1996; Rappuoli and Covacci, 1996) to amplify different loci spread over the cag I region. Thus, *cagA* and *cagE* represented cagI. These primer pairs were designed on the basis of published sequences (Tomb et al., 1997; Akopyants et al., 1998; van Doorn et al., 2000). *H. pylori* strains ATCC 43526 and ATCC 43504 which have been determined to have the entire cag PAI, was used as positive controls and ATCC 51932 as a negative control as it lacks *cagA* for each experiment. PCR amplification specificity for the different loci was assessed by testing *H. pylori* strains ATCC 43526, ATCC 43504 and ATCC 51932 with each experiment. Amplification by PCR was performed in a volume of 25 µL containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5-2.5 mmol/L $MgCl_2$, 200 µmol/L deoxynucleoside triphosphates, 2 units Taq DNA polymerase (Promega) and 25 µmol of both forward and reverse primers (synthesized by MWG Automatic synthesizer). The total volume was made up with double distilled water. The amplified products were resolved on a 2% agarose gel in 1× TAE buffer (40 mM Tris-acetate-1 mM EDTA (pH 8.0) containing ethidium bromide (0.5 µg/ml) and then visualized under UV light. PCR was performed in a Perkin Elmer 9700 thermal cycler (Applied Biosystems).

Cytokine assays

Cell culture supernatants were harvested and analyzed for cytokines by ELISA (Enzyme linked immunosorbent assay) techniques with commercially available kits. Human IL-8 kits were obtained from BD OptEIA. Cytokine assay was calibrated against the World Health Organization international standards by the kit manufacturer. The lower limit of detection for the IL-8 assay was 0.8 pg/ml.

IL-8 expression by AGS cells

To analyze whether herbs could prevent the *H. pylori*-induced IL-8 expression, AGS cells were pretreated with the herbs prior to *H. pylori* infection. AGS cells were treated with herb extracts in cell culture medium before *H. pylori* infection. The treated cells were then infected with 10 clinical isolates that were *cagA and cagE* gene positive and one *cagA* and *cagE* gene negative *H. pylori* strain. The supernatants were collected and stored at −80°C before analysis. The level of IL-8 in supernatants from AGS cell cultures was determined by using a ELISA (sandwich enzyme-linked immunosorbent assay) kit (BD systems), according to the manufacturer's instruction.

Statistical assessment

The statistical package for social science - SPSS (Release 17, standard version, copyright © SPSS; 1989-2010) was used for data analysis. The descriptive analysis was done for demographic and clinical features. Results were presented as mean ± standard deviation for quantitative variables and number (percentage) for qualitative variables. Differences in proportion were assessed by using Pearson Chi square; Fisher exact or likelihood ratio test where appropriated. The kappa (κ) test was done to test agreement between sensitivity to herb extracts and cagA-PAI genes e.g. *cagA* and *cagE*. P value less than 0.05 was considered as statistical significant, all 'p' values were two sided. Non parametric, Mann Whitney test was used to compare two variables while one-way analysis of variance was used to compare negative and positive controls with effect of different extracts on expression of IL-8 by AGS cell.

RESULTS

H. pylori strains were sensitive to CLR in 61(81%) and resistant in 14(19%). They were sensitive to AMX in

Table 2. Correlation of sensitivity of *Helicobacter pylori* to herbs and antibiotics.

	Clarithromycin			Amoxicillin		
	Sensitivity	**Resistance**	**Kappa (P value)**	**Sensitivity**	**Resistance**	**Kappa (P value)**
			***A. sativum* (5.5 mg/ml)**			
Sensitive	36(59)	4(29)	0.193(0.04*)	39(57)	1(17)	0.124(0.06)
Resistant	25(41)	10(71)		30(43)	5(83)	
			***A. sativum* (11 mg/ml)**			
Sensitive	42(69)	4(29)	0.285(0.005*)	45(65)	1(17)	0.177(0.02*)
Resistant	19(31)	10(71)		24(35)	5(83)	
			***P. nigrum* (10 mg/ml)**			
Sensitive	13(21)	2(14)	0.031(0.55)	14(20)	1(17)	0.007(0.83)
Resistant	48(79)	12(86)		55(80)	5(83)	
			***P. nigrum* (20 mg/ml)**			
Sensitive	13(21)	2(14)	0.031(0.55)	14(20)	1(17)	0.007(0.83)
Resistant	48(79)	12(86)		55(80)	5(83)	
			***C.cyminum* (8 mg/ml)**			
Sensitive	14(23)	1(7)	0.07(0.18)	14(20)	1(17)	0.007(0.83)
Resistant	47(77)	13(93)		55(80)	5(83)	
			***C. cyminum* (16 mg/ml)**			
Sensitive	21(34)	2(14)	0.09(0.14)	22(32)	1(17)	0.034(0.44)
Resistant	40(66)	12(86)		47(68)	5(83)	
			Herb mix (23.5 mg/ml)			
Sensitive	19(31)	3(21)	0.47(0.47)	20(29)	2(33)	-0.01(0.82)
Resistant	42(69)	11(79)		49(71)	4(67)	
			Herb mix (47 mg/ml)			
Sensitive	26(43)	4(29)	0.076(0.33)	28(41)	2(33)	0.018(0.73)
Resistant	35(57)	10(71)		41(59)	4(67)	

Results were presented as mean ± standard deviation for quantitative variables and number (percentage) for qualitative variables. Differences in proportion were assessed by using Pearson Chi square, Fisher exact or likelihood ratio test where appropriated. The kappa (κ) test was done to test agreement between two variables. *P value less than 0.05 was considered as statistical significant, all *p* values were two sided

69(92%) and resistant in 6(8%).

Comparison of sensitivity of *H. pylori* isolates to herbs and antibiotics

The sensitivity of the *H. pylori* strains to *A. sativum* (garlic) 5.5 mg/ml was 59% (36/61) (p=0.04) and *A. sativum* 11 mg/ml was 69% (42/61) (p=0.005) compared to CLR, respectively. The sensitivity of the *H. pylori* strains to *A. sativum* 5.5 mg/ml was 57% (39/69) (p=0.06), and *A. sativum* 11mg/ml was 65% (45/69) (p=0.02) compared to AMX, respectively (Table 2).

Correlation of sensitivity of *H. pylori* isolates to herbs and antibiotics

There was a border line correlation between *H. pylori* isolates sensitivity to low concentration of *A. sativum* in a concentration of 5.5 mg/ml and AMX (ҁ= 0.124, p=0.06) while there was a significant correlation between *A. sativum* 11 mg/ml and AMX (ҁ= 0.177, p=0.02). There was a significant correlation between *H. pylori* sensitivity

to both concentration of *A. sativum* and CLR (ĸ= 0.193, p=0.04) and (ĸ= 0.285, p=0.02), respectively (Table 2).

Distribution of *cagA*-pathogenicity island genes

In these *H. pylori* strains *cagA* was positive in 42(72%) and *cagE* gene in 30(52%) (Table 3).

Effect of herbs related to *cagE* gene

When *H. pylori* isolates had *cagE* gene (n=30), 37% (11/30) (p=0.02) they were sensitive to herb mix 23.5 mg/ml, 60% (18/30) (p<0.001) to herb mix 47 mg/ml and 43% (13/30) (p=0.04) to *C. cyminum* 16 mg/ml, respectively (Table 3). When *H. pylori cagE* gene was negative, *A. sativum* 11 mg/ml 14/15(93%) sensitivity correlated with AMX that is, 14/21(67%) (ĸ=0.407, p=0.016) and 15/15 (100%) compared to CLR 15/25 (60%) (ĸ=0.243, p=0.05).

Effect of herb extracts on AGS cells viability

Assays were performed to assess the cell viability using trypan blue staining of untreated as well as herb treated AGS cells. At their maximum tested concentration, the extracts of *A. sativum*, *C. cyminum*, and *P. nigrum* have no significant effect upon AGS cell viability.

Effect of herbs on IL-8 expression by AGS cells

The expression of IL-8from AGS cells was not inhibited by pretreatment with herbs. Treatment of *A. sativum* was associated with least IL-8 secretion by the *H. pylori* infected gastric epithelial cell compared to *P. nigrum*, *C. cyminum* and herb mix (Table 4).

Comparison of effect of herbs extracts on IL-8 expression by AGS cells

The secretion of IL-8 by AGS cells infected with *H. pylori* strains (positive control) demonstrated a significant increase in the IL-8 level compared to AGS cell alone (negative control) (P= 0.0002, Figure 1). IL-8 secretion in the AGS cell treated with garlic when infected with *H. pylori* strains demonstrated a decrease in IL-8 compared to positive control (P=0.052, Figure 2). There was no difference in the IL-8 secretion when AGS cell treated with *C. cyminum* (*P*=0.580, Figure 3), *P. nigrum* (*P*=0.105, Figure 4), and extracts mixture (*P*=0.2799, Figure 5) were infected with *H. pylori* strains in comparison to positive control. There was a significant difference in the secretion of IL-8 between the positive and negative controls with AGS cells treated with herb extract mix, (P=0.0037, Figure 6), *C. cyminum*, (P<0.0001, Figure 7), *P. nigrum* (P=0.0046, Figure 8) and *A. sativum* (P=0.0021, Figure 9), respectively.

DISCUSSION

In this study, *H. pylori* strains demonstrated an *in vitro* sensitivity to CLR and AMX in over 80% of the cases. *H. pylori* strains sensitivity to *A. sativum* (garlic) was concentration dependent. It was not so in case of *P. nigrum*, *C. cyminum* and their mix. When *cagE* gene was positive, it made the isolates increasingly sensitive to herb mix in tested concentrations. In contrast when *cagE* gene was negative, *H. pylori* strains were still sensitive to *A. sativum* which was similar to AMX and CLR. The viability of the gastric epithelial cells following exposure to the different extracts and their mix was found intact. Incubation of AGS cells with *H. pylori* increased IL-8 secretion. It was the least expressed in the presence of *A. sativum* compared to *P. nigrum*, *C. cyminum* and their mix. Previously, some other indigenous medicinal plants such as *Alpinia galangal*, *Cinnamomum cassia*, etc were also reported to demonstrate strong inhibitory activity against IL-8 secretion from *H. pylori* infected gastric epithelial cells (Zaidi et al., 2012). *C. cassia* suppressed TNF-α stimulated IL-8 secretion in a concentration-dependent-manner from *H. pylori* infected gastric epithelial cells. *Curcumin*, a local spice-derived polyphenol, suppressed *H. pylori*-induced NF-kappaB activation (Zaidi et al., 2009). In a recent study, methanol and acetone extracts from *Acacia nilotica* and *Calotropis procera* demonstrated anti-*H. pylori* activity stronger than metronidazole and comparable with tetracycline (Amin et al., 2013).

It is known that production of IL-8 by *H. pylori* is associated with the release of soluble bacterial extracellular factors or to direct contact with bacterial surface components. Cytokines in the gastric environment provide communication between mucosal macrophages and gastric epithelial cells and thus may regulate the degree of inflammation and epithelial cell degeneration in *H. pylori* infection (Benabdelmoumene et al., 1991; Birkholz et al., 1993). The proinflammatory cysteine protease caspase-1 is autocatalytically activated upon cytosolic sensing of a variety of pathogen-associated molecular patterns by Nod (nucleotide-binding oligimerization domain)-like receptors. Active caspase-1 processes pro-IL-1β and pro-IL-18 to generate the bioactive cytokines to initiate pathogen-specific immune responses. The Nod-like receptors as pattern recognition receptors activate inflammasomes in macrophages. Inflammasomes are essential for the activation of inflammatory caspases and subsequent maturation of their pro-inflammatory cytokine substrates and induction of pyroptosis (Khare et al., 2010). Caspase-1 is activated and IL-1β and IL-18 are processed *in vitro* and *in vivo* as

Table 3. *Helicobacter pylori* and herbs relation to *cagA* and *cagE* genes.

Herb	*CagA*			*CagE*		
	Positive	Negative	P	Positive	Negative	P
***Allium sativum* (5.5 mg/ml)**						
Sensitive	22(52)	6(38)	0.311	17(57)	11(39)	0.18
Resistant	20(48)	10(62)		13(43)	17(61)	
***Allium sativum* (11 mg/ml)**						
Sensitive	26(62)	8(50)	0.41	19(63)	15(54)	0.45
Resistant	16(38)	8(50)		11(37)	13(46)	
***Piper nigrum* (10 mg/ml)**						
Sensitive	7(17)	3(19)	0.85	6(20)	4(14)	0.56
Resistant	35(83)	13(81)		24(80)	24(86)	
***Piper nigrum* (20 mg/ml)**						
Sensitive	7(17)	3(19)	0.85	6(20)	4(14)	0.56
Resistant	35(83)	13(81)		24(80)	24(86)	
***Cuminum cyminum* (8 mg/ml)**						
Sensitive	7(17)	3(19)	0.85	6(20)	4(14)	0.73
Resistant	35(63)	13(81)		24(80)	24(86)	
***Cuminum cyminum* (16 mg/ml)**						
Sensitive	13(31)	5(31)	0.98	13(43)	5(18)	0.04*
Resistant	29(69)	11(69)		17(57)	23(82)	
Mix (23.5 mg/ml)						
Sensitive	10(24)	4(25)	0.92	11(37)	3(11)	0.02*
Resistant	32(76)	12(75)		19(63)	25(89)	
Mix (47 mg/ml)						
Sensitive	16(38)	6(37)	0.97	18(60)	4(14)	>0.001*
Resistant	26(62)	10(63)		12(40)	24(86)	

Results were presented as mean ± standard deviation for quantitative variables and number (percentage) for qualitative variables. Differences in proportion were assessed by using Pearson Chi square, Fisher exact or likelihood ratio test where appropriated. P value less than 0.05 was considered as statistical significant, all *p* values were two sided.

Table 4. Effect of Herbs on IL-8 expression by AGS cells in the presence of Helicobacter pylori infection

Parameter	*Allium sativum* + Hp + AGS	*Cuminum cyminum* + Hp + AGS	*Piper nigrum* + Hp + AGS	Mix 2 + Hp + AGS	AGS alone (negative control)	AGS plus Hp (positive control)
Mean	106	121	117	133	91	152
Standard deviation	71	82	77	71	47	61

a consequence of *H. pylori* infection. Caspase-1 activation and IL-1 signaling are required for the efficient control of *Helicobacter* infection. The processing and release of a regulatory caspase-1 substrate, IL-18, counteracts the proinflammatory activities of IL-1β and serve to reduce excessive gastric immunopathology (Hitzler et al., 2012). *H. pylori* causes gastric epithelial cell damage and atrophy via oxidative stress and the type

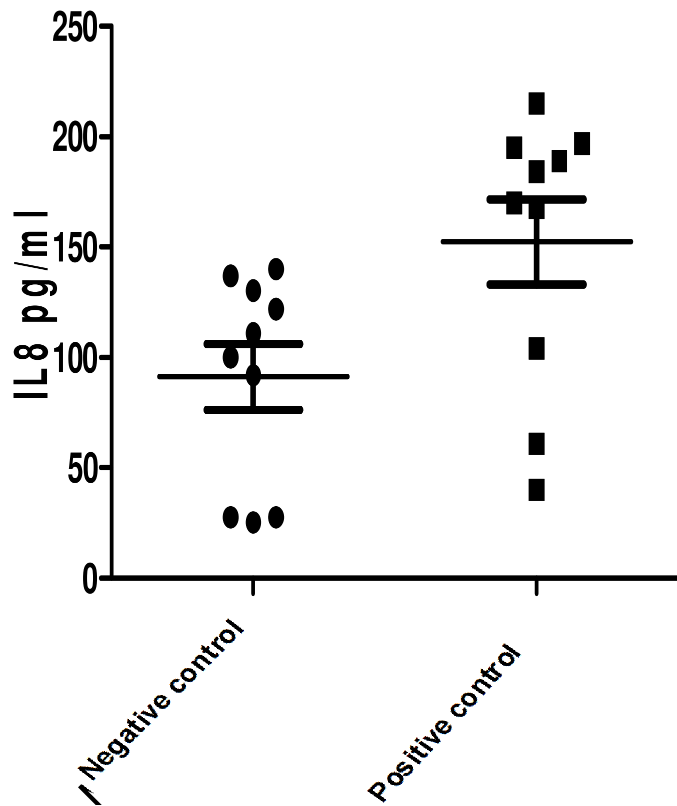

Figure 1. AGS cells not exposed to *H. pylori* isolates and exposed (Mann-Whitney test p = 0.0211).

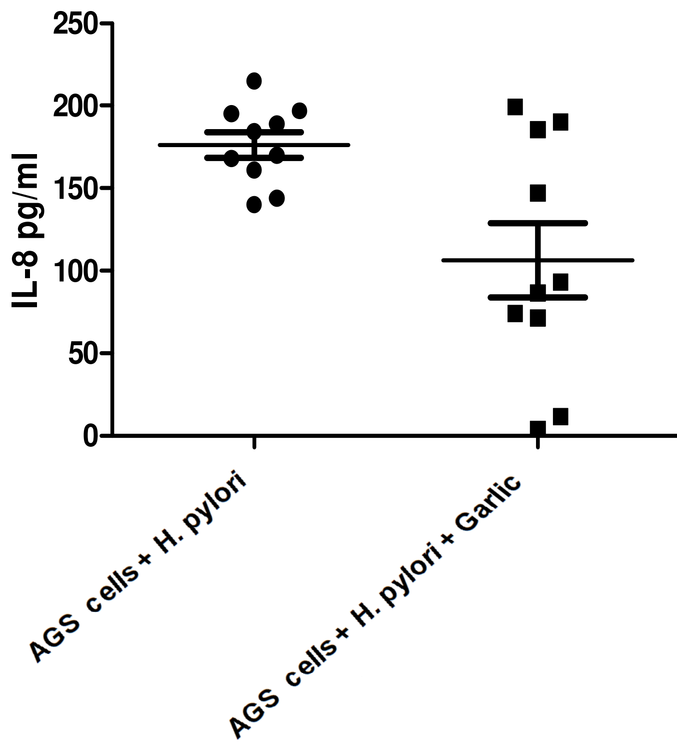

Figure 2. AGS cells and *H. pylori* isolates with and without Garlic (Mann-Whitney test p = 0.0524).

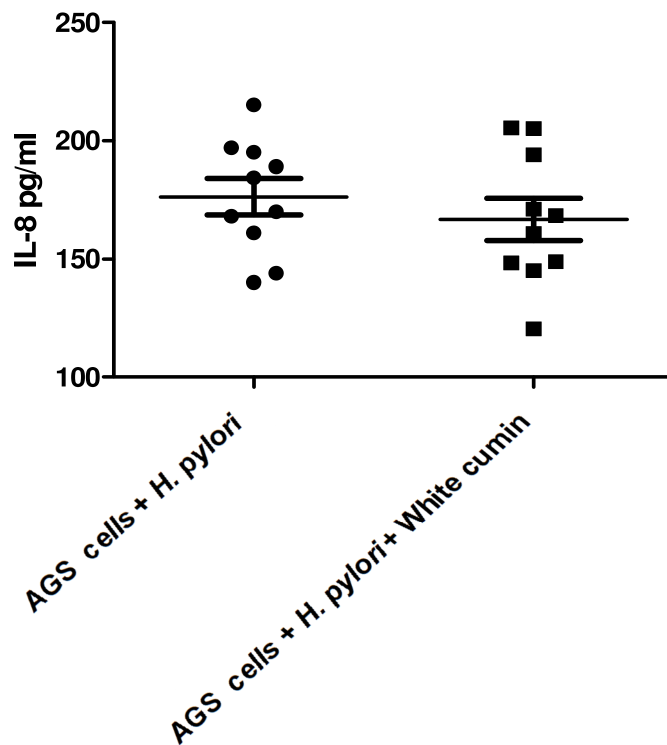

Figure 3. AGS cells and *H. pylori* isolates with and without White cumin (Mann-Whitney test p = 0.5787).

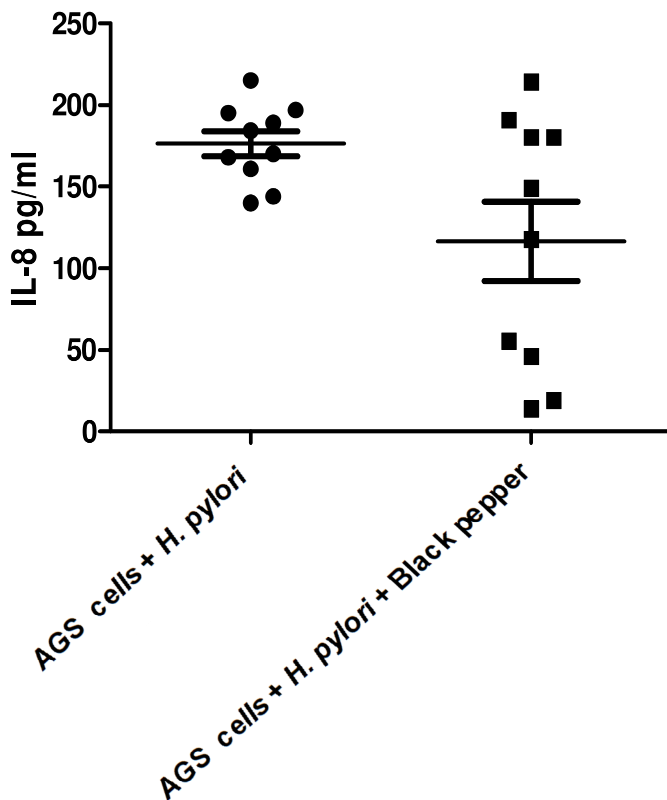

Figure 4. AGS cells with *H. pylori* isolates alone and with Black pepper extract (Mann-Whitney test p = 0.1051).

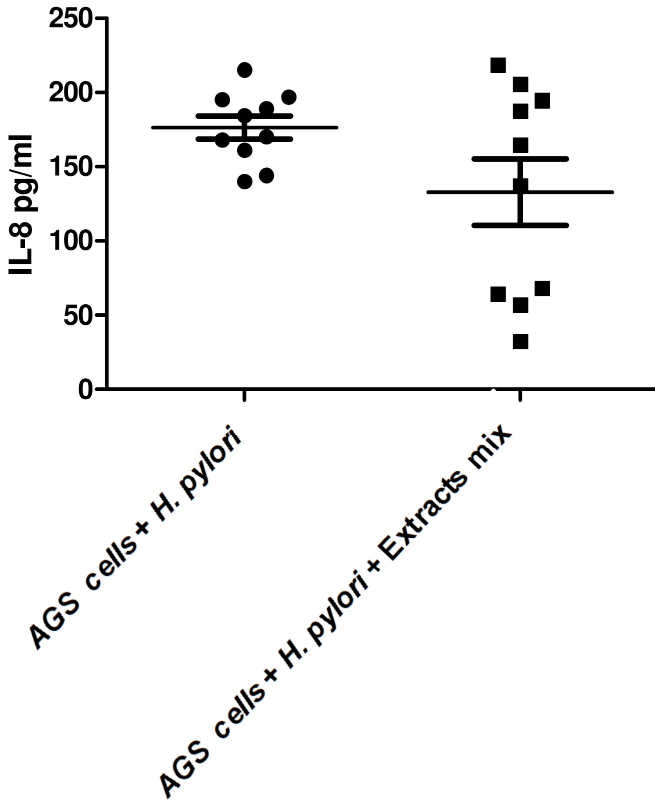

Figure 5. AGS cells and *H. pylori* isolates with and without Extract mix (Mann-Whitney test p = 0.22799).

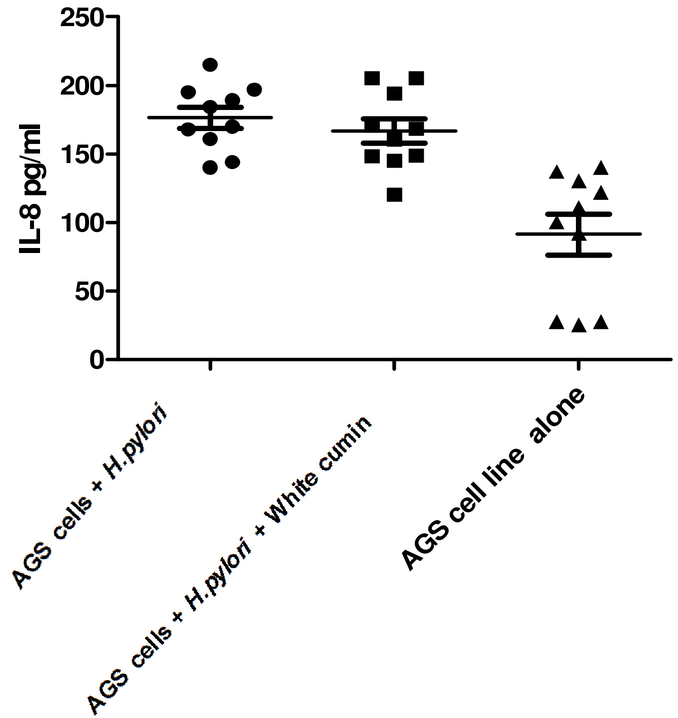

Figure 7. AGS cells alone and with *H. pylori* isolates and White cumin using One-Way Analysis of Variance ($p < 0.0001$).

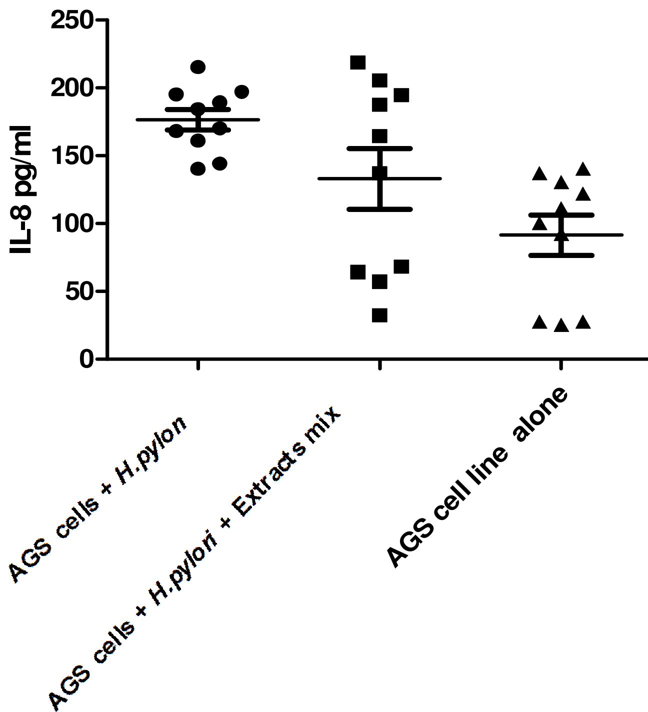

Figure 6. AGS cells with *H. pylori* isolates and Extract mix using One-Way Analysis of Variance (p = 0.0037).

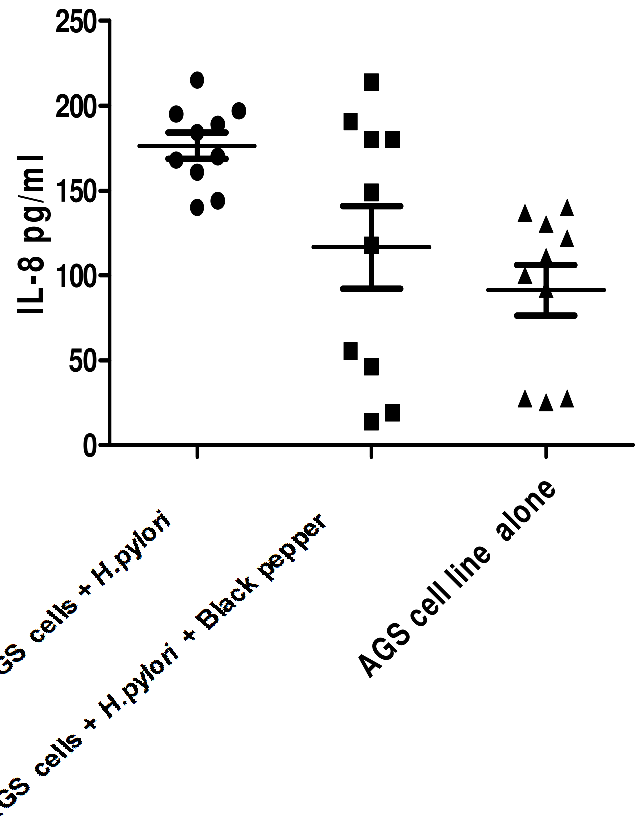

Figure 8. AGS cells alone and with *H. pylori* isolates and Black pepper using One-Way Analysis of Variance (p = 0.0046).

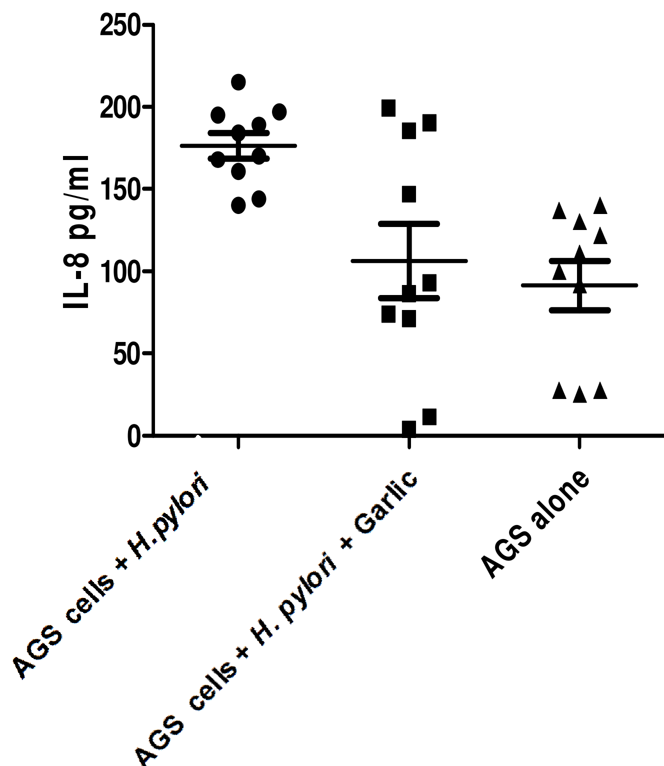

Figure 9. AGS cells alone and with *H. pylori* isolates and Garlic using One-Way Analysis of Variance (p = 0.0125).

I apoptotic or type II autophagic programmed cell death-related pathway. Catechins are well described poly-phenols (Graham, 1992). Their major active component, epigallocatechin-3-gallate (EGCG) possesses antibac-terial activity against *H. pylori* by inhibiting urease and vacuolating cytotoxinA activity (Mabe et al., 1999; Matsubara et al., 2003; Ruggiero et al., 2006; Yang et al., 2008).

The attenuation of the IL-8 production by *A. sativum* pretreatment might contribute to prevention of IL-8-induced inflammatory response. *A. sativum* 1, 2-vinyldithiin (1,2-DT) and thiacremonone inhibit the activity of inflammatory messenger molecules. The inflammatory transcription factor NF-kappaB gets inhibited while the release of inflammatory messaging molecules IL-6 and IL-8 by macrophage cells is reduced in white adipose tissue (Ban et al., 2009). This study suggests that ingestion of these plant extracts could have therapeutic implications for patients with *H. pylori* induced gastritis and duodenal ulcer. If given orally, they would be able to affect *H. pylori* despite the short amount of time they remain in the stomach during digestion. These plants extracts can be used in combination with antibiotics, possibly increasing the rate of eradication, as has been shown *in vitro* for cranberry juice (Shmuely et al., 2004). Most studies have used plant extracts that are obtained by chemical processing, e.g., ethanol extracts. The usage of these processing methods may not be freely accessible compared to these plant extracts. This study has demonstrated that plant extracts are effective against *H. pylori.* Using mixture of these herbal extracts will provide a simple treatment, which will be relatively inexpensive and could be incorporated into the diet of the patient. In general herbal treatments are usually unregulated and the safety of plants consumed is often unknown. The extracts of plants used and shown to be effective in this study are already commonly consumed, and thus they are known to be safe. The validation in human beings with well-designed clinical trial is to follow.

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NON-STANDARD ABBREVIATIONS

AMX, amoxicillin; **CLR,** clarithromycin; **EGCG,** epigallocatechin-3-gallate; **NF-κB,** the nuclear factor kappa B

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