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DETERMINATION OF IN-VITRO AND IN-VIVO ACTIVITIES OF ALOE VERA. L AGAINST H. PYLORI

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ABSTRACT

Background Antimicrobial and antiulcer activities of *Aloe vera* plant extract were evaluated against *H. pylori* strains. According to several studies, oral consumption of *Aloe Vera* works effectively to soothe conditions like heartburn, arthritis and rheumatism pain and asthma. Researchers are now investigating the new roles of antimicrobial and antiulcer activities of *Aloe vera* in acid peptic disorders. Therefore the current study is aimed to evaluate the anti-*H. pylori* and antiulcer properties of *Aloe vera*.

Methods

The extracts of *Aloe vera* plants were harvested and stored for the *in-vitro* and *in-vivo* studies. The antimicrobial activity was detected by using disc diffusion method. *In vivo* activities were also studied in albino rats by ethanol induced ulcers and the treatment regimens.

Results

The results showed that *Aloe vera* exhibited strong antimicrobial activity against *H. pylori* at two different concentrations of 250, 500µg/mL in comparison with standard Clarithromycin. *In vivo* studies showed a very good response in ulcer healing properties. Study found that use of *Aloe vera* may act as complementary and alternative medicine for gastrointestinal diseases.

INTRODUCTION

Aloe barbadensis miller commonly called as *Aloe vera or Curacao aloe* is used antimicrobial agent against bacteria, fungi and viruses. Anti bacterial properties of *A. vera* is well studied in *Staphylococcus spp* and *Candida spp* (1, 2). In addition, the extracts of *A. vera* also exhibits strong anti-inflammatory and immunomodulatory effects. *A. vera* juice is considered helpful for relieving many types of gastrointestinal irritation and also commonly used for treating gastro esophageal reflux diseases (GERD) (3, 4). *H. pylori* is a human pathogen that causes chronic gastritis, has a role in gastric and duodenal ulcer, is involved in gastric carcinogenesis and the bacteria have been classified as a definite (Class I) carcinogen to humans (5). This gram –ve gastric pathogen is also regarded as a possible important factor in at least a subset of patients with functional dyspepsia (6, 7, 8, 9). There are many studies both *in vitro* and *in vivo* which have effectively demonstrated antibacterial activities of many active principles such of ginger, thyme, evodia, berberine, and curcumin. However

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there are only few studies which have demonstrated antibacterial activity of Indian *A. vera* extracts *in vitro*. Recently, Heron *et al* (2009) reported that the extracts of *A. vera* possess very strong therapeutic effect on *H. pylori* (10, 11, 12). These results showed a significant *in vitro* effect of *A. vera* against *H. pylori* that could be considered a valuable support in the treatment of the infection and may contribute to the development of new and safe agents for inclusion in anti-*H. pylori* treatment regimens. Although several traditionally used herbs are reported to possess anti-ulcer and anti-*H. pylori* effects, scientific validation and the mechanism by which they induce such effects are yet to be identified. In view of this, *A. vera* known for its antibacterial activities was used in the present study to evaluate its ulcer healing and anti-*H. pylori* effects in Albinorats. In essence, the present study deserves special considerations in view of the holistic approach in the management of peptic ulcer disease and *H. pylori*.

Materials and Methods

Collection and Preparation of extracts of Aloe vera

Leaves of *Aloe vera* were collected and inner and outer coat were separated in sterile conditions. The outer coat of the leaf was homogenized aseptically, lyophilized and stored in 4^0C for *in-vivo* studies. The lyophilized powder was dissolved in DMSO overnight, and the mixture was centrifuged at 3,000 rpm for 10 min and the supernatant was used for the experiment.

In-vitro culturing of H. pylori

Stock cultures of *H. pylori* were used for the secondary culturing and lawn preparation (GC-16, MS-5). Loop full of inoculum was streaked on to the surface of Brucella agar medium (Difco Laboratories, Detroit, Michigan, USA) supplemented with 7% sheep blood and containing the antibiotics Vancomycin (6 mg/mL), Amphoteracin B (2 mg/mL) and Polymixin B (2500 units/mL). This inoculum was performed within 1 hr after the sampling. The agar plates were incubated at 37^0 C for 3-7 days under microaerophilic conditions. The culture was considered to be positive for *H. pylori* by observing small, translucent, tiny colonies.

Disk diffusion method

H. pylori strains isolated from different subjects suffering from gastrointestinal disorders were used for evaluating the susceptibility of *Aloe vera*. Previously reported Modified Kirby Bauer's disk diffusion assay was used for *in vitro* evaluation (13, 14).

Minimum Inhibitory Concentration (MIC)

MIC determination was used for the detection of anti-*H. pylori* activity in *Aloe vera*. This study was designed to evaluate the MICs of *Aloe vera* along with common antibiotics like amoxicillin and clarithromycin against *H. pylori* isolates. The results were expressed as the MICs of all the isolates were showed in the **Table 1**

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Table .1

Data showing zone of inhibition of H. pylori incubated at variable concentration of Alo vera by disk diffusion method (Data are expressed as the means ± SD of three replicates).

Concentration	Diameter of the Zone of inhibition (mm) ± SD	Remarks
5µg/mL	12± 1	Mild response
10µg/mL	16 ± 4	Mild response
50µg/mL	20 ±3	Moderate response
100µg/Ml	22 ± 3	Moderate response
250µg/mL	25 ±6	Strong response
500µg/mL	32 ±5	Strong response

Anti- H. pylori and Anti-ulcer activity of Aloe vera in AlbinoRats

Animals and Storage

Male Albinorats weighing 200-250gm were used for assessing and evaluation of the anti-*H. pylori* and anti-ulcer activities. Animals were housed at 22 ± 2^0 C under a 12-hour light /12- hour dark cycle *Ad libitum* access to water and food before experimentation. Prior to the studies, the rats were fed overnight with water provided and *Ad libitum* (Asakawa A *et al*., 2003) (15).

Ethanol induced Ulcer and H. pylori infection

The albino rats were divided randomly and 5 groups of 6 animals each and starved for 24 hours but had free access to water. Water was however withdrawn 2 hours before experiments. One ml of absolute ethanol was administered orally to all the groups. After one hour of ethanol administration, the animals were sacrificed under anesthesia. The stomach was isolated, opened along the greater curvature and washed. Macroscopic examination of the stomachs of the animals in all the groups was done. The presence of ulcers was counted using a magnifying glass. The diameter of the ulcers was measured using a vernier caliper and scored on scale. The procedure was repeated after the 2 weeks of treatment regimen. The ulcer inductions were calculated as per the calculations described previously (16).

Histopathology

After sacrificing the rats the gastric biopsies were collected in 10% buffered formalin and these tissues were embedded in paraffin blocks for

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histopathological analysis. Approximately 3-4µm thick paraffin sections were subsequently stained with haematoxylin and eosin (H&E) stain and observed under microscope. A blinded pathologist examines the slides for the presence of *H. pylori* and the rate of inflammation and histological lesions were graded using updated Sydney system of classification (Dixon *et al*., 1996) (17).

Treatment Strategies

Lyophilized *A. vera* extracts were diluted DMSO and used for the treatment schedules. Albinorats were arranged four different groups as per the study design and treatment schedules.

Group 1 served as control and received normal saline, Groups 2, 3 and 4 received 50, 100 and 500 mg/kg of the *Aloe vera* extract respectively by oral intubations, while group 5 received 100 mg/kg triple therapy orally.

Efficacy and Safety observations

The rats will be observed for their characteristic changes and toxicity after treated with *Aloe vera*. One rat from each group was sacrificed at 2 weeks after the bacterial inoculation and remaining rats were sacrificed 3weeks after treatment with *Aloe vera*. Their stomachs will be dissected and the mucosal surface of each stomach was gently scraped and then placed into 1mL of PBS. 100µL of the suspension of the homogenized stomach was diluted with 1 or 5mL of PBS and then 100µL of the diluted suspension was spread on agar plate. The colonies of *H .pylori* in the homogenate of the stomach will be counted and expressed as log10CFU/stomach. Further the stomach biopsies will be collected for determination of ulcer index, histopathology and status of *H. pylori* infection respectively.

Statistical Analysis

Data analyses were done using different statistical software's. Student's *t* test and ANOVA one-way analysis were performed for the analysis of the data. "*P*" value "< 0.05" is considered to be statistically significant.

RESULTS

Mild response was observed at concentrations 5 and 10µg/mL, moderate response at 50 and 100µg/mL concentration of *A. vera* was observed however strong response was observed at 250 and 500mg/mL concentrations. The inhibition zone increased with the concentration of *A. vera* increased; PBS as a negative control did not inhibit the growth of *H. pylori*. As the compound was active against *H. pylori*, figure 1 shows the killing curves of the compound at different concentrations ranging from 10 to 500 µg/mL were evaluated. The initial concentrations of *A. vera* 10 and 50 µg/mL, initial inhibition in the growth of *H. pylori* was observed up until 10 h, following which an increase in the viability of the bacteria was observed. The compound was found to completely inhibit the growth of *H. pylori* at concentrations of 100 and 250 µg/ml at 16 h of incubation; this decrease was seen at 8 h, when the concentration was increased to 500µg/ml. The time course lethal effect of the compound on *E. coli* was studied at 10 µg/mL and was found to inhibit the growth of *E. coli* after 1 h of incubation. Clarithromycin used as a reference antibiotic showed inhibitory activity against *H. pylori* after 4 h of incubation with complete inhibition seen at 12 h of incubation. **Table 2** showed the MIC values of *A. vera* at different concentrations. We observed a concentration range of 1.5 to 14µg/mL of *A. vera* in MIC values against *H. pylori*. *In vivo* studies using Wistar rats revealed that the ulcer indexes in *H. pylori* infected rats were decreased with the post treatment

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with *A. vera.* As the concentration of the *A. vera* increased, the ulcer index also decreased **(Table 3)**. Almost 80% of the total stomach area was ulcerated when infected with *H. pylori*, which was decreased to almost 20% with 500mg/mL concentration of *A. vera*, which revealed the ulcer healing activity of *A.vera*. However, *A. vera* itself in ethanol treated rats also showed 23% ulcer index, which reveals that *A. vera* was having the direct effect on *H. pylori* as well as on ulcer, thus *A. vera* revealed both anti-*H. pylori* and anti-ulcer activity.

Aloe vera works without toxicity or allergic effects because its nutrient and water content act as buffers. Therefore, the theory of synergistic relationship (all chemical and physical components of the plant work together to add up to a greater benefit than the sum total of each individual item) is one which is supportable by both history and science. Recent researches indicating that orally administered *Aloe vera* preparations have the capacity to cure chronic venous leg ulcers (20). From these *in-vivo* studies Table 2 revealed that there are significant changes in the ulcers and antimicrobial activity of *Aloe vera*.

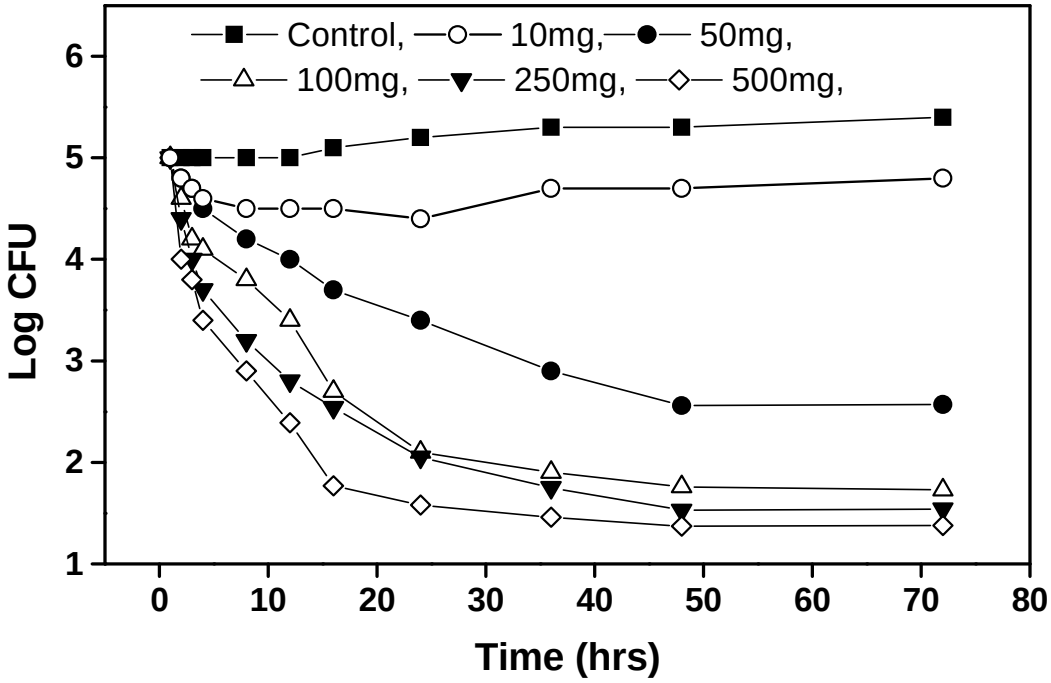

Fig. 1

Time course viability studies of Aloe vera at different concentrations with H. pylori (pH 7.0).

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Table .2
Showing the MIC values of *Aloe vera* against *H. pylori*

Compound	MIC (µg/mL)
Clarithromycin	1.1
*A.vera*1	15
*A.vera*2	11
*A.vera*3	8
*A.vera*4	6
*A.vera*5	2

Table .3
Shows the effect of *Aloe vera* or Clarithromycin treatment on Ethanol treatment and *H. pylori* induced ulcer in different groups.

Group (10 rats in each group)	Treatment regimen	Ulcer Index (%)= Ulcerated area/Total Stomach area x 100 ± SD	P value
Group 1	Control	0	
Group 2	Ethanol treated	72± 7	<0.001
Group 3	Ethanol treated & *H. pylori* infected	80± 9	<0.001
Group 4	Ethanol treated & *H. pylori* infected + Aloe vera (10µg/mL) treated	66± 13	0.04
Group 5	Ethanol treated & *H. pylori* infected + Aloe vera (50µg/mL) treated	55±17	0.02
Group 6	Ethanol treated & *H. pylori* infected + Aloe vera (100µg/mL) treated	41± 11	0.005
Group 7	Ethanol treated & *H. pylori* infected + Aloe vera (250µg/mL) treated	30±09	<0.001
Group8	Ethanol treated & *H. pylori* infected + Aloe vera (500µg/mL) treated	19±08	<0.001
Group 9	Ethanol treated + *H. pylori* infected + Clarithromycin	20± 04	0.002
Naproxen control (4 rats)	Ethanol treated + Aloe vera (500µg/mL) treated	22±11	0.01

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DISCUSSIONS

Peptic ulcer disease in conjunction with *H. pylori* infection is a worldwide problem and the cost of eradicating it using standard antibiotic regimen is also high. Therefore exploration of alternate regimens based on herbal medicine would not only provide major boost to combat multi-drug resistant *H. pylori* but will also provide a simple, inexpensive means to resolve this global health hazard. To combat the threat of these antibiotic resistant *H. pylori*, several medicinal plants are been increasingly used owing to their antibacterial properties. Recent studies revealed that extracts of *Aloe vera* contains a heat stable substance with possible therapeutic potential. There are many studies both *in vitro* and *in vivo* which have effectively demonstrated antibacterial activities of ginger, thyme, evodia, berberine, and curcumin extracts (Smith-Palmer *et al*., 1998). However there are only few studies which have demonstrated antibacterial activity of *Aloe vera* extracts against *H. pylori* infection.

Aloe vera belonging to the lily family and related to the onion, garlic and asparagus, from 2100 BC onwards evidence of using of aloe dating as was discovered on a Mesopotamian clay tablet (Cairo 1862). The results show a significant *in-vitro* effect of *Aloe vera* against *H. pylori* that could be considered a valuable support in the treatment of the infection and may contribute to the development of new and safe agents for inclusion in anti-*H. pylori* treatment regimens. The plant is used extensively in the preparation of many ayurvedic formulations for a variety of health concerns including chronic ulcers, fungal skin infections, digestive problems, constipation, heart disease, tumors, nervous disorders, and asthma. Even though the *in vitro* results look quite provocative, however the ulcer healing and act against *H. pylori in vivo* needs further and rigorous investigation using more number of animal models followed by human clinical trials to provide more conclusive evidence of their safety and efficacy before marketing.

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