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Antiulcer Activity Aloe Vera Gel And Its Interaction With Conventionally Used Antiulcer Drug Pantoprazole In Rats

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ABSTRACT

Aloe vera are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing, or soothing properties. There is, however, little scientific evidence of the effectiveness or safety of *Aloe vera* extracts for either cosmetic or medicinal purposes, and what positive evidence is available is frequently contradicted by other studies. *Aloe vera* has been used as a popular folk medicine. It is a colourless mucilaginous gel obtained from the parenchymatous cells in the fresh leaves of *Aloe vera.* It has been claimed that *Aloe vera* gel has the ability to cure gastric ulcers or protect against its formation in both animals and humans. This study evaluates the effect of *Aloe vera* gel on gastric and duodenal ulcers and determines its interaction with conventionally used antiulcer drugs; pantoprazole. The antiulcer activity of *Aloe vera* gel at three different doses and its interaction with Pantoprazole was evaluated in, pylorus ligation induced gastric ulcer, stress induced gastric ulcer and indomethacin induced gastric ulcer models. In all these models, the common parameter determined was ulcer index. All the three doses of *Aloe vera* gel produced marginal ulcer healing effect and augmented the ulcer healing effect of pantoprazole in stress and Indomethacin induced gastric ulcers, while in pylorus ligated rats, *Aloe vera* gel demonstrated gastric anti-secretory and antiulcer effect. *Aloe vera* gel possesses dose dependent antiulcer effect and augments the antiulcer action pantoprazole in rats.

Keywords: *Aloevera* gel, gastric cytoprotection, gastric ulcer, pantoprazole.

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INTRODUCTION

Peptic ulcer diseases (PUD) are the disorder of the gastrointestinal system and millions of people suffer from this disease globally. The main causes of peptic ulcers are hyperacidity, excessive intake of alcohol, overeating, high intake of spicy foods, food poisoning, high intake of coffee, smoking, NSAIDs, stress, presence of helicobacter pylori. Several gastric anti-secretory and cytoprotective drugs are available in the market for the treatment of PUD and none of them are free from side effects. Since modern drugs are not able to wholly cure chronic diseases but, rather, to prevent further deterioration associated with them, patients must take drugs for extended periods of time. Due to the ineffectiveness as well as the potential side effects, patients are often led to explore complementary or alternative medicines such as herb, and medicinal botanicals in addition to modern drug[2]. However, simultaneous administration of herbs and drugs may mimic, magnify or oppose the pharmacological effects of each other. This Phenomenon is called as Herb-drug interactions. It is widely believed that although herbs hold promise as therapeutically effective medicaments, in-depth and appropriate studies should be carried out to confirm their efficacy in the presence of modern medicines. *Aloe vera* has been used as a popular folk medicine. It is a colourless mucilaginous gel obtained from the parenchymatous cells in the fresh leaves of *Aloe vera* (family- *Aloeaceae*). The positive effects are thought to be due to the presence of compounds such as polysaccharides, mannans, anthraquinones and lectins.

Various studies have revealed that *Aloe vera* leaf skin *(AVLS*) possesses many pharmacological activities, including purgative, antifungal, antibacterial, anticancer, immunomodulatory, and antioxidant, antidiabetic, wound healing property. It was proposed that the *A. vera* gel containing products improved skin hydration possibly by means of a humectants mechanism.

Aloe vera has been proved to be very effective drug in reducing inflammation. It has been claimed that *Aloe vera* gel has the ability to cure gastric ulcers or protect against its formation in both animals and humans. The anti-ulcer activities of *Aloe vera* has been attributed to several possible mechanisms including its anti-inflammatory properties, healing effects, mucus stimulatory effects and regulation of gastric secretions. The present study determines the effect of *Aloe vera* gel on gastric and duodenal ulcers and evaluates its interaction with conventional antiulcer drug pantoprazole by using experimental models of gastric ulcers in rats[1-4].

MATERIALS AND METHOD

DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma Aldrich), Potassium chloride (Qualigens Fine Chemicals, Mumbai, India), Sodium chloride (Merck Specialities Private Limited, Mumbai,

India), Ethyl acetate (S D Fine Chemicals, Mumbai, India), Folin ciocalteau reagent(Phenol reagent), Ketamine (Prem Pharmaceutical Ltd, Indore, India), Magnesium sulphate (Nice Chemicals Pvt Ltd, Cochin, India), Nitro blue tetrazolium(NBT) (Loba Chemicals, Mumbai, India).

Experimental Animals:

Albino rats of either sex weighing 150-200 g were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol (KCP/IAEC-16/2011-12).

Preparation of *Aloe* extract and dose selection. [5-13]

Fresh *Aloe vera* leaves were collected from healthy plants. The rind was selectively removed and the colourless parenchyma was grounded in a blender and was centrifuged. The supernatant was lyophilized and stored at 4^0C.Known amount of lyophilized powder was extracted with 95% ethanol and nearly 85% of the solvent used was recovered by distillation over the boiling water bath at atmospheric pressure and the remaining under reduced pressure in rota vapor. A known amount of solvent free extract was suspended in water to obtain the desired concentration of the *Aloe vera* leaf gel. Dose was selected based on acute oral toxicity study performed according to the OPPTS guidelines (Office of Prevention, Pesticide and Toxic Substance) following the limit test procedure. The animals were fasted over night prior to the experiment. Test dose of 2 g/kg, 3 g/kg and 5 g/kg were given orally to mice. Low doses were found to be safe. Hence $1/10^{th}$, $1/20^{th}$ and $1/50^{th}$ of the minimum safe dose corresponding to 150 mg/kg,100 mg/kg and 50 mg/kg orally were selected as high, Intermediate and low doses respectively.

Preliminary Phytochemical Analysis

The juice was subjected to qualitative phytochemical analysis to determine the presence of alkaloids, carbohydrates, proteins, steroids, triterpenoids, tannins and flavonoids.

Determination of Antioxidant Effect

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method. The diluted working solutions of the *Aloe vera* gel extracts were prepared in methanol. Gallic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample

solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below.

Percent (%) inhibition of DPPH activity A-B/B ×100

Where A = optical density of the blank and B = optical density of the sample.

By using IC_{50} value the antioxidant activity of *Aloe* extract preparation were tested.

Experimental groups

The rats will be grouped as follows

Group 1: Control

Group 2: Low dose of *Aloe vera* leaf gel extract (50mg/kg) (LALGE)

Group 3: Intermediate dose of *Aloe vera* leaf gel extract (100mg/kg) (IALGE)

Group 4: High dose of *Aloe vera* leaf gel extract (150mg/kg) (HALGE)

Group 5: Pantoprazole (20mg/kg p.o) (PZL)

Group 6: Low dose of *Aloe vera* leaf gel extract (LALGE) plus PZL

Group 7: Intermediate dose of *Aloe vera* leaf gel extract (IALGE) plus PZL

Group 8: High dose of *Aloe vera* leaf gel extract(HALGE) plus PZL.

EXPERIMENTAL MODELS[14-26]

Pylorus ligation induced ulcers

The procedure of Shay *et a1*, with modifications described by Kulkarni was used. Animals were fasted for 36 hr before pylorus ligation with water *ad libitum* by placing them individually in cages to avoid coprophagy and cannibalism. Normal saline (1ml/rat p.o) was administered twice daily to all the animals during the fasting period. Under ketamine HCl (100 mg/kg, i.m) & xylazine HCl (16 mg/kg, i.m) anesthesia, the abdomen was opened by midline incision below the xiphoid process. The pyloric portion of stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal wall was closed with sutures. *Aloe extract* of 50 mg/kg, 100 mg/kg & 150 mg/kg (p.o), & Pantoprazole (20mg/kg, p.o) or vehicle was administered intra duodenally immediately after pylorus ligation. The animals were deprived of food and water during the postoperative period and were sacrificed 6 hr after pylorus ligation by over dose of ether anesthesia. The stomach was isolated and the contents of the stomach were collected and centrifuged. The gastric juice was used for estimation of free acidity(R), total acidity(R), & mucin content(R). The stomachs were cut open along the greater curvature and the ulcer score and ulcer index was determined using the formula.

The ulcers were given scores based on their intensity as follows 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer.

Ulcer index = 10/X

Where X = total mucosal area/ total ulcerated area

Cold restraint stress induced ulcers

The ulcer was induced by subjecting the animals to cold restraint stress. *Aloe extract* of (50 mg/kg, 100 mg/kg & 150 mg/kg p.o.) & (200 mg/kg p.o.) & pantoprazole (20mg/kg p.o.) were administered 30 minutes prior to subjection of stress. The animals were placed in a restraint cage and the cage was placed at a temperature of 20˚C for 2-3 hours. After 3 hours the animals were sacrificed by over dose of ether anesthesia and the stomach was isolated and cut opened along the greater curvature. The ulcer index and ulcer score was determined.

Healing of indomethacin induced gastric ulcers, a model to study free radical scavenging activity

The gastric ulcers were induced by administering indomethacin (8mg/kg. p.o.) for 5 days during this period the animals were fed normally. The animals were then treated with pantoprazole (20 mg/kg p.o.) and *Aloe extract* (50mg/kg,100 mg/kg p.o.& 150 mg/kg p.o.) once daily for 5 days after induction of ulcer while the control group received only vehicle. The last dose of indomethacin was considered as 0th day. Rats were sacrificed on the 0th day and 5th day, 6 hours after the last dose of the drug treatment. The stomachs were removed and they were cut open along the greater curvature and ulcer score and ulcer index were determined. The glandular portion of the stomach was taken and was used for estimation of mucin content, total proteins, antioxidant factors like super oxide dismutase activity, and catalase activity.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test. For comparing nonparametric ulcer scores, ANOVA followed by non-parametric Dunn post-test was used. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Preliminary Qualitative photochemical analysis of *Aloe vera* gel revealed the presence of reducing sugars, proteins, amino acid, triterpenoids and flavonoids. Antioxidant test of *Aloe vera* showed that the IC_{50} of *Aloe vera* gel was 220 µg/ml and that of gallic acid was 6 µg/ml. The *Aloe vera* gel extract was evaluated for its antiulcer activity in combination with pantoprazole using different animal models like pylorus ligation induced gastric ulcer model, healing of

indomethacin induced gastric ulcers and stress induced ulcers. All the treated groups have showed significant reduction in gastric ulcers.

The present work showed the effect of *Aloe vera* on gastric ulcer and also its interactive effect with antiulcer drug Pantoprazole on gastric secretion, gastric ulcer healing. Stress induced ulcer are probably mediated by histamine release, which in turn increases gastric secretion & cause disturbance of gastro mucosal microcirculation, alteration in motility & reduced production of mucus. *Aloe vera* possesses healing effects, mucus stimulatory effects and regulation of gastric secretions may helpful in reducing the gastric ulcers or the reduction may be due to local effect on gastric motility or gastric secretion. The agents having cytoprotective effect are effective in preventing the ulcers induced by indomethacin. The anti ulcer activity of *Aloe vera* is due to its anti inflammatory, cytoprotective, healing, anti oxidant and mucus stimulatory effect. The extract was also effective in increasing gastric mucus content and activities of endogenous antioxidant enzymes. Hence, it can be suggested that cytoprotective effect in Indomethacin induced gastric ulcers may be due to both increase in cytoprotective mucus secretion and antioxidant property. *Aloe vera* extract was effective in all the tested models of gastric ucers. *Aloe vera* contains a number of Phenol, flavonoids, calcium and many other chemical constituents, which may be responsible for the anti ulcer activity. The result of the present study suggests that *Aloe vera* can enhance the anti-ulcerative property of conventional drugs like Pantoprazole.

Table 1: Effect of *Aloe*extract on ulcer index, ulcer score, free acidity, total acidity and mucin content.

Treatment	Ulcer index	Ulcer score	Free acidity	Total acidity	Mucin content
Control	0.39±0.05	14.2±1.1	46.7±2.2	57±1.9	8.9±0.96
LALGE(50mg/kg)	0.27±0.09^{a}	12.3±0.78^{a}	43.3±1.9	51.3±2.32^{a}	12.6±0.86^{a}
IALGE(100mg/kg)	0.21±0.012^{b}	10.2±0.98^{a}	31.4±0.99^{a}	31.3±0.98^{b}	17.5±1.2^{a}
HALGE(150mg/kg)	0.15±0.005^{b}	7.3±1.76^{b}	15.4±0.87^{b}	16.1±1.28^{b}	28.6±1.2^{b}
PZL(20mg/kg)	0.19±0.009^{b}	8±0.52^{b}	5.43±0.27^{b}	10.7±0.87^{b}	31.1±4^{b}
LALGE+PZL	0.16±0.008^{b*}	5.9±0.67^{b}	1.93±0.56^{b}	5.8±0.19^{b*}	39.5±2.9^{b*}
IALGE+PZL	0.14±0.002^{b**}	3.72±0.48^{b*}	1.65±0.09^{b}	3.8±0.23^{b**}	46.5±1.6^{b**}
HALGE+PZL	0.11±0.006^{b**}	1.91±0.09^{b**}	0.9±0.06^{b*}	2.56±0.5^{b}	49.6±1.45^{b**}

All values are mean ± SEM (n=6), $^{a}P<0.1$, $^{b}P<0.01$, $^{c}P<0.001$ when compared with Control, $^{*}P<0.1$, $^{**}P<0.01$, $^{***}P<0.001$when compared with PZL.

Table 2: Effect on mucin content, ulcer index, ulcer score, total proteins, anti oxidant factors in indomethacin induced ulcers

Treatment	Ulcer index	Ulcer score	Mucin content(µg/ gm)	Total proteins (mg/ml)	SOD Units/mg of proteins
CONTROL	1.37±0.006	14.5±0.42	0.59±0.02	18.9±0.9	3.2±0.32
LALGE(50mg/kg)	1.31±0.003^{b}	12.1±0.3^{b}	0.9±0.04^{a}	24.5±1.10^{a}	4.1±0.34
IALGE(100mg/kg)	0.64±0.01^{b}	7.6±0.42^{b}	1.2±0.04^{b}	28.08±0.9^{b}	5.2±0.12^{a}
HALGE(150mg/kg)	0.4±0.006^{b}	5.83±0.47^{b}	1.5±0.05^{b}	33.5±1.6^{b}	7.1±0.29^{b}
PZL(20mg/kg)	0.46±0.004^{b}	6.16±0.47^{b}	1.45±0.06^{b}	30.7±1.9^{b}	8.2±0.72^{b}
LALGE+PZL	0.22±0.01^{b}*	2.6±0.21b**	1.9±0.05b*	34.6±1.4^{b}	9.9±0.69^{b}
IALGE+PZL	0.09±0.008b**	1.01±0.36b**	2.36±0.05b**	36.2±2.1b**	11.7±0.09b*
HALGE+PZL	0.035±0.002b**	0.6±0.01b**	2.7±0.3b**	38.6±0.85b**	16.6±0.58b**

All values are mean ± SEM (n=6), a $p< 0.1$, b $P <0.01$, c $P <0.001$ when compared with control, *$p< 0.1$, ** $P <0.01$, *** $P <0.001$ when compared with Pantoprazole.

Table 3:Effect of *Aloe* extracts on ulcer index and ulcer score in stress induced gastric ulcers.

Treatment	Ulcer index	Ulcer score
CONTROL	0.9±0.09	16±1.1
LALGE(50mg/kg)	0.83±0.07	
IALGE(100mg/kg)	0.7±0.045^{a}	10±0.91^{a}
HALGE(150mg/kg)	0.42±0.009^{b}	7±1.12^{b}
PZL(20mg/kg)	0.34±0.06^{b}	6.6±0.9^{b}
LALGE+PZL	0.18±0.005^{b}	5.6±0.81^{b}
IALGE+PZL	0.12±0.01^{b*}	
HALGE+PZL	0.075±0.005^{b**}	2.3±0.26^{b*}

All values are mean ± SEM (n=6), a $p< 0.1$, b $P <0.01$, c $P <0.001$ when compared with control, *$p< 0.1$, ** $P <0.01$, *** $P <0.001$ when compared with Pantoprazole.

CONCLUSION

The *Aloe vera* extract and its interaction with Pantoprazole shows effective in decreasing the development of gastric ulcer in stress induced gastric ulcers, indomethacin induced gastric ulcers and pylorus ligation gastric ulcers. The high dose and interaction of *Aloe* with pantoprazole shows more effective in entire ulcer induced models when compared to control. The antiulcer effect of *Aloe vera* may be due to the reduction in gastric acid secretion, gastric cytoprotective activity and mucus secretory activity.

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