

Effect of watery juice of *aloe vera* and *opuntia ficus* leaves on gastric acid secretion and healing of acute gastric ulcer in rats

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Abstract: This study aimed to reveal the effect of oral administration of *aloe vera* and *opuntia ficus* watery juice on gastric acid secretion and healing of acute gastric ulcer in rats. The experiment was performed on 49 rats distributed into 7 equal groups. One group of rats was kept as a negative control while, the other 6 groups were orally given 10% aspirin solution (200 mg/Kg b.wt.) on an empty stomach (fasting period of 18 hrs.) for induction of acute gastric ulcer. One group of rats with experimental gastric ulcer was kept as a positive control, while the other groups were orally given *aloe vera*, *opuntia ficus* and their combination as a watery juice daily for 2 weeks. At the end of experiment, all rats were sacrificed and volume, total acidity and pepsin concentration in the gastric juice were determined. Gastric ulcer indexes were calculated and the histopathology of stomachs was carried out. Results showed that oral administration of the watery juice of *aloe vera* in combination with *opuntia ficus* at 20 % for 2 weeks to rats with experimental ulcer caused significant antisecretory and anti-ulcer effects. The combined juice of both plants decreased volume and total acidity of the gastric juice and inhibited pepsin outflow. It also increased in curative ratio from gastric ulcer and ameliorated of histopathological lesions caused by aspirin. Therefore, intake *aloe vera* combined with *opuntia ficus* at 20 % for 2 weeks as a drink with a sweet substance may be useful in the management of gastric ulcer. The study recommends conducting this experiment on patients suffering from acute gastric ulcer. [Journal of American Science 2010;6(10):600-605]. (ISSN: 1545-1003).

Keywords: *Aloe vera*, *Opuntia ficus*, Gastric secretion, Gastric ulcer, Histopathology, Rats.

1. Introduction

Peptic ulcers are erosions in the lining epithelium of the stomach (gastric ulcers) or the duodenum (duodenal ulcers). On contrary to popular belief, ulcers are not caused by spicy food but are most commonly due to an infection by *Helicobacter pylori* (*H. pylori*) bacteria, which are now recognized as the primary cause for most ulcers. The second most common cause of ulcers is long-term use of non steroidal anti-inflammatory drugs as aspirin (Khayyal, *et.al.* 2001).

Botanical materials have been traditionally used by herbalists for the prevention and treatment of peptic ulcers. Botanical compounds with anti-ulcer activity include flavonoids (e.g. quercetin, naringin, and anthocyanosides) saponins; (from *Panax japonicus* and *Kochia scoparia*); tannins (from *Linderae umbellatae*); gums and mucilages (e.g. myrrh). Among plant kingdom, some plants such as liquorice (Dehpour *et.al.*, 1995, Langmead and Rampton, 2001 and Adel *et. al.*, 2004); *aloe vera* (Suvitayavat *et. al.*, 2003); capsicum or chilli (Yeoh *et. al.*, 1995; Teng *et. al.*, 1998 and Borrelli and Izzo, 2000); cabbage (Noumi and Dibakto, 2000) and ginger (Chaudhuri, *et. al.*, 2004) have been used extensively and their clinical efficacy was documented. The plant kingdom might provide a useful source of new anti-ulcer compounds (Borrelli and Izzo, 2000).

This study aimed to reveal the effect of oral administration of *aloe vera* and *opuntia ficus* watery juices alone and in combination, on gastric acid secretion and healing of acute gastric ulcer induced by aspirin in male rats.

2. Material and Methods

Plants:

Fresh green leaves of *Aloe vera* (Family *Liliaceae*) and *Opuntia ficus* (Family *Cactaceae*) were obtained from the farm of Agricultural research Center, ministry of Agriculture, Giza, Egypt. The leaves were authenticated in the Botany Department, Faculty of Agriculture, Cairo University.

Aspirin (Acetyl salicylic acid, Aspegic ®):

It was obtained in the form of 1 gm vials from Ameriya Company for Pharmaceutical and Chemical Industries, Cairo, Egypt.

Rats:

Male mature albino rats (175 – 185 gm b.wt and 12 – 14 weeks age) of Sprague Dawley strain were obtained from the Laboratory Animal Colony, Helwan, Egypt. Rats were kept in plastic cages under strict hygienic conditions and fed on the basal diet for one week before start of the experiment for acclimatization. Water was provided *ad libitum*.

Preparation of basal diet:

The basal diet was prepared according to Reeves *et. al.* (1993). It is consisted of 20 % protein (casein), 10% sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers. The remainder was corn starch upto 100 %.

Preparation of watery juice of the plants:

The fresh green leaves (pads) of *aloe vera* and *opuntia ficus* were incised several times longitudinally in the middle using a sharp cutter. The released viscous substance was collected in 250 ml glass beaker. To 150 gm of this substance, 600 ml distilled water were added and mixed in a blender for 15 minutes to obtain 20 % watery juice which kept in a refrigerator till use.

Experiment and grouping of rats:

Forty nine male albino rats were divided into two main groups; the first main group (7 rats) was fed on the basal diet and kept as a negative control (C-ve). The second main group (42 rats) was orally given aspirin by stomach tube; aspirin was freshly prepared by dissolving one vial (1 gm) in 10 ml distilled water to obtain 10% solution. Aspirin solution was orally given to the rats on an empty stomach at a dose of 200 mg/Kg b.wt. for induction of acute gastric ulcer according to the method described by Agrawal *et. al.* (2000). Rats with gastric ulcer were distributed into six subgroups (n=7 rats) and received for 2 weeks the following treatments:

- Subgroup 1: None treated (positive control group, C +ve).
- Subgroup 2: Given orally 10% *aloe vera* watery juice.
- Subgroup 3: Given orally 20% *aloe vera* watery juice.
- Subgroup 4: Given orally 10% *opuntia ficus* watery juice.
- Subgroup 5: Given orally 20% *opuntia ficus* watery juice.
- Subgroup 6: Given orally 10% *aloe vera* + 10% *opuntia ficus* watery juice.

Determination of Gastric ulcer index:

On the last day of experiment period, all rats were fasted for 18 hours and in morning of the next day all rats were sacrificed and their stomachs were ligated around both openings (cardiac and pyloric). Each stomach was injected by 2ml distilled water and the gastric juices were collected in petri dishes after removal of the ligature around one opening and then their volume was measured. The stomachs were then opened longitudinally, washed with saline solution and examined using a magnifying lens. The length of gastric ulcer was measured and expressed as ulcer index (UI).

The curative ratios from the ulcer were calculated for the treated groups according to the method described by Akhtar and Ahmad (1995) using the following equation:

$$\text{Curative Ratio} = \frac{\text{LUC} - \text{LUT}}{\text{LUC}} \times 100$$

Where:

LUC = length of ulcer in the positive control group.

LUT = length of ulcer in the treated group.

Measurement of volume of gastric juice:

Gastric juices from all groups were collected in test tubes, centrifuged at 5000 r.p.m. for 10 minutes and their volume of were measured by a graduated cylinder. Percentages of the decrease in volume of the gastric juice of the treated groups compared to the positive (C+ve) control group were calculated according to the method described by Agrawal *et. al.* (2000) using the following equation:

$$\text{Percentage of the decrease} = \frac{\text{VJC} - \text{VJT}}{\text{VJC}} \times 100$$

Where:

VJC = Volume of gastric juice of the positive control group

VJT = Volume of gastric juice of the treated group.

Determination of total acidity in gastric juice:

Total acidity of the collected gastric juices was determined by titration of gastric juice with 0.1N sodium hydroxide solution (NaOH). In a conical flask, 1ml of the gastric juice was added to 10 ml distilled water and 1 ml of oxalic acid. Two drops of phenolphthalein as indicator were added to the flask. NaOH solution was dropped from a graduated burette till the end point (pink color) is reached. The volume of NaOH solution required to produce neutralization was measured and the total acidity was determined according to the method described in A.O.A.C. (1995). Percentages of the decrease in total acidity of gastric juice of the treated groups compared to the positive (C+ve) control group were calculated using the following equation:

$$\text{Percentage of the decrease} = \frac{\text{TAC} - \text{TAT}}{\text{TAC}} \times 100$$

Where:

TAC = Total acidity of gastric juice of the positive control group

TAT = Total acidity of gastric juice of the treated group.

Determination of pepsin concentration in gastric juice:

Concentrations of pepsin (a proteolytic enzyme that degrades dietary protein) in the collected

gastric juices were measured chemically using spectrophotometer at 313 nm according to the method described by Schniath (1989).

Histopathological examination:

Stomachs of the sacrificed rats were taken and immersed in 10% formalin solution. The specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Dehydrated specimens were cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Heamtoxylin and Eosin stain for histopathological examination according to the method described by Carleton (1979).

Statistical analysis:

Data were presented as means \pm SE and statistically analyzed using one-way ANOVA offered by Duncan's multiple range tests according to Snedecor and Cochran (1986). $P < 0.05$ was used to indicate significance.

3. Results and Discussion

The obtained results are recorded in Tables (1) to (4) and shown in Figs.(1) to (5). Results revealed that oral administration of *aloe vera* watery juice at 10 and 20 % to male rats with gastric ulcer significantly ($P < 0.05$) decreased the volume of gastric juice by 31.7 and 35.6%, respectively as compared to the control positive group. *Opuntia ficus* watery juice at 10 and 20 % when given orally to rats with gastric ulcer decreased the volume of gastric juice by 12.15 and 25.50 % respectively. The combined juice of *aloe vera* and *opuntia ficus* significantly ($P < 0.05$) decreased the volume of gastric juice by 57.25% compared to the positive control group as depicted in Table (1).

The decrease in volume of gastric juice caused by *aloe vera* juice reported in the present study agreed with the findings of Borrelli and Izzo (2000); Suvitayavet *et al.* (2003), Yusuf *et al.* (2004) and Kallaya *et al.* (2006). The previous authors reported that *aloe vera* juice decreased the volume of gastric juice and produced an antiulcer activity in rats. Concerning *opuntia ficus* watery juice, the decrease in volume of gastric juice caused by its administration to rats was in agreement with that obtained by Galati, *et al.* (2003). The authors reported that *opuntia ficus* juice inhibited the ulcerogenic activity of ethanol in treated rats and examination by light microscopy showed restoration of the normal gastric mucosal architecture. The high decrease in volume of gastric juice caused by juice of both of *aloe vera* and *opuntia ficus* in the treated rats may be attributed to the additive effect of both juices. The antisecretory and acid lowering effects of *aloe vera* and *opuntia ficus*

were previously reported by Noumi and Dibakto (2000) and Borrelli and Izzo (2000) in rats.

Table (1): Effect of oral administration of *aloe vera*, *opuntia ficus* watery juice and their combination on volume of the gastric juice. (n= 7 male)

Groups and treatments	Volume of gastric juice (ml)	
	Mean \pm SE	Decrease (%)
1- Control (C-ve)	1.07 \pm 0.04 ^e	---
2- Aspirin (C+ve)	2.55 \pm 0.02 ^a	---
3- <i>Aloe vera</i> juice 10%	1.74 \pm 0.01 ^c	31.7
4- <i>Aloe vera</i> juice 20%	1.64 \pm 0.03 ^c	35.6
5- <i>Opuntia ficus</i> juice 10%	2.24 \pm 0.02 ^b	12.15
6- <i>Opuntia ficus</i> juice 20%	1.90 \pm 0.01 ^c	25.50
7- Combined juice 20 %	1.09 \pm 0.04 ^d	57.25

Means with different superscript letters differ significantly ($P < 0.05$) using ANOVA test.

Table (2): Effect of oral administration of *aloe vera*, *opuntia ficus* watery juice and their combination on total acidity of the gastric juice. (n=7 male)

Groups and treatments	Titratable acidity of gastric juice (mg equiv./L)	
	Mean \pm SE	Decrease (%)
1- Control (C-ve)	1.140 \pm 0.03 ^d	---
2- Aspirin (C+ve)	2.361 \pm 0.02 ^a	---
3- <i>Aloe vera</i> juice 10%	1.399 \pm 0.05 ^b	40.7
4- <i>Aloe vera</i> juice 20%	1.349 \pm 0.03 ^b	42.88
5- <i>Opuntia ficus</i> juice 10%	1.591 \pm 0.02 ^c	32.6
6- <i>Opuntia ficus</i> juice 20%	1.422 \pm 0.04 ^c	39.8
7- Combined juice 20 %	1.150 \pm 0.01 ^d	51.3

Means with different superscript letters differ significantly ($P < 0.05$) using ANOVA test.

As shown in Table (2) oral administration of *aloe vera* juice at 10 and 20 % or *opuntia ficus* juice at 10 and 20% to rats with aspirin induced gastric ulcer decreased the total titratable acidity (TTA) of gastric juice by 40.7 and 42.88% or by 32.6 and 39.8%, respectively. The combined juice of *aloe vera* and *opuntia ficus* caused a decrease in TTA of gastric juice by 51.3% as compared the control positive group. Similar findings were previously reported by Borrelli and Izzo, (2000), Yusuf *et al.* (2004) and

Kallaya *et al.* (2006) for *aloe vera* juice and by Park *et al.* (1998) and Galati, *et al.* (2003) for *opuntia ficus* juice.

Chemical analysis of gastric juice of rats given orally the watery juice of *aloe vera* at 10 and 20 % or *opuntia ficus* at 10 and 20% revealed significant decreases in pepsin concentration by 9.87 and 24.30% or by 8.10 and 17.72%, respectively as compared to the positive control group. The combined juice of both plants at 20% decreased pepsin concentration by 46.07% as recorded in Table (3). Similar results were obtained by Saito *et al.* (1989) and Yusuf *et al.* (2004) for *aloe vera* juice and by Galati, *et al.* (2003) for *opuntia ficus* juice.

Data recorded in Table (4) show that the gastric ulcer index in male rats with experimental gastric ulcer (positive control) was 8.45 ± 0.24 mm compared to zero (no ulcer) in the negative control group (normal rats). Oral administration of *aloe vera* and *opuntia ficus* watery juice to male rats for 2 weeks resulted in a decrease in gastric ulcer index and an increase in the curative ratio from gastric ulcer. The curative ratios from ulcer were 27.2; 42.0; 32.5; 36.6 and 56.8 % for 10 and 20% of *aloe vera* and 10 and 20% of *opuntia ficus* respectively.

The ulcer healing effect was greater by use of the combined juice of both *aloe vera* and *opuntia ficus* at 20 %. The reported anti-ulcer effect of *aloe vera* juice was similar to that previously obtained by Noumi and Dibakto (2000), Suvitayavat *et al.* (2003), Yusuf *et al.* (2004) and Kallaya *et al.* (2006) in rats who concluded that *aloe vera* promote gastric ulcer healing. The authors attributed the anti-ulcer effect of *aloe vera* juice to its acid lowering effect and antiinflammatory activity. The most beneficial ulcer healing activity was reported by administration the combined juice of both *aloe vera* and *opuntia ficus* and this could be explained by their additive effect.

Histopathological examination of stomachs of the negative control (normal) rats revealed normal histology of gastric mucosa as shown in Fig. (1). Stomachs of the positive control rats (given aspirin) showed focal necrosis, desquamation of gastric mucosa and leucocytic cells infiltration (Fig.(2) Examined sections of stomachs of the rats given 20% *aloe vera* watery juice showed only mild necrotic changes of gastric mucosa (Fig. 3). Examined sections of the stomachs of rats given 20% *opuntia ficus* watery juice showed mild necrotic changes in gastric mucosa and submucosal edema as shown in Fig (4). In rats given 20% *aloe vera* and *opuntia ficus* watery juice, the examination revealed normal histology of stomach as shown in Fig.(5).

Results of histopathological examination of stomachs of rats, reported in this study, confirm the finding of in vivo anti-secretory and ulcer healing

activity of *aloe vera* and *opuntia ficus* watery juice. The cytoprotective effect of *aloe vera* on gastric mucosa could be partly due to its anti-inflammatory effect that reported by Langmead *et al.* (2004) or due to the antioxidant effect of anthocyanins content of *aloe vera* as mentioned by Bolkent (2004). Concerning *opuntia ficus*, Galati, *et al.* (2003) reported that *opuntia ficus* juice produced antioxidant and antiulcerogenic activities. It also caused restoration of the normal gastric mucosal architecture in rats with experimental gastric lesions. The authors attributed the antiulcerogenic effect of *opuntia ficus* to presence of mucilage content in its juice.

Table (3): Effect of oral administration of *aloe vera*, *opuntia ficus* watery juice and their combination on pepsin concentration in gastric juice. (n=7 male)

Groups and treatments	pepsin concentration (mg/ml)	
	Mean \pm SE	Decrease (%)
1- Control (C-ve)	20.9 \pm 1.5 ^d	---
2- Aspirin (C+ve)	39.5 \pm 3.3 ^a	---
3- <i>Aloe vera</i> juice 10%	35.6 \pm 2.4 ^b	9.87
4- <i>Aloe vera</i> juice 20%	29.9 \pm 1.8 ^c	24.30
5- <i>Opuntia ficus</i> juice 10%	36.3 \pm 1.6 ^b	8.10
6- <i>Opuntia ficus</i> juice 20%	32.5 \pm 1.4 ^b	17.72
7- Combined juice 20 %	21.3 \pm 1.7 ^c	46.07

Means with different superscript letters differ significantly (P = 0.05) using ANOVA test.

Table (4): Effect of oral administration of *aloe vera*, *opuntia ficus* watery juice and their combination on gastric ulcer index. (n= 7 male)

Groups and treatments	Gastric ulcer index	Curative ratio (%)
	Mean \pm SE (mm)	
1- Control (C-ve)	---	---
2- Aspirin (C+ve)	8.45 \pm 0.24 ^a	---
3- <i>Aloe vera</i> juice 10%	6.15 \pm 0.18 ^b	27.2
4- <i>Aloe vera</i> juice 20%	4.90 \pm 0.21 ^c	42.0
5- <i>Opuntia ficus</i> juice 10%	5.70 \pm 0.14 ^c	32.5
6- <i>Opuntia ficus</i> juice 20%	5.35 \pm 0.18 ^c	36.6
7- Combined juice 20 %	3.65 \pm 0.18 ^d	56.8

Means with different superscript letters differ significantly (P = 0.05) using ANOVA test.

In conclusion, oral administration of watery juice of *aloe vera* and *opuntia ficus* at 20% for 2 weeks to rats with aspirin induced - gastric ulcer produces antisecretory, cytoprotective and gastric ulcer healing activities. The major goal for treating patients with peptic ulcer disease is to avoid the extreme elevation of gastric acid secretion and the direct irritation of gastric mucosa.

Therefore, intake of *aloe vera* and *opuntia ficus* juice as a drink with a sweet substance may be useful for peptic ulcer patients. This study

recommends conducting this experiment on patients suffering from gastric ulcer disease.

Cross sections in stomachs of rats showing:

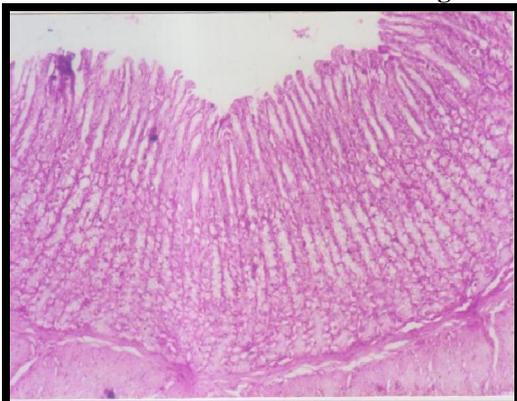


Fig. (1) Stomach of - ve control (normal) rat showing normal histology of stomach. (H and E x 200)

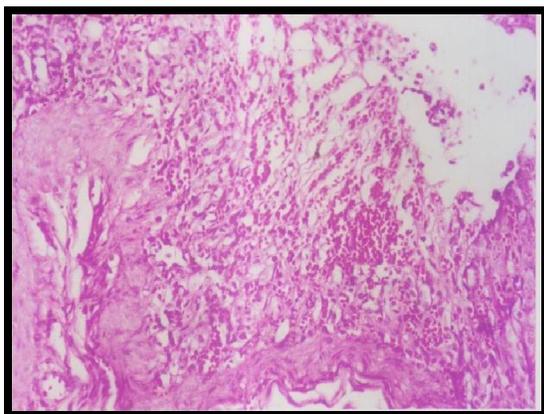


Fig. (2) Stomach of +ve control rat showing focal necrosis, desquamation of gastric mucosa and leucocytic cells infiltration. (H and E x 100)

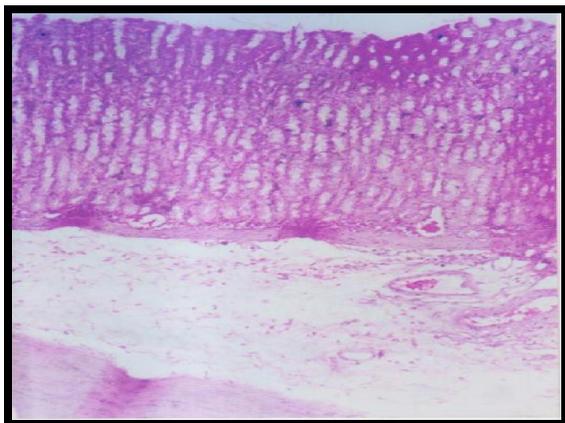


Fig. (3) Stomach of a rat given 20% *aloe vera* watery juice showing only mild necrotic changes gastric mucosa. (H and E x 200)

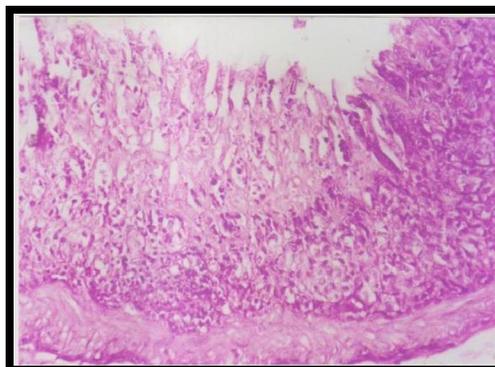


Fig. (4) Stomach of a rat given 20% *opuntia ficus* watery juice showing mild necrotic changes and submucosal edema. (H and E x 100)

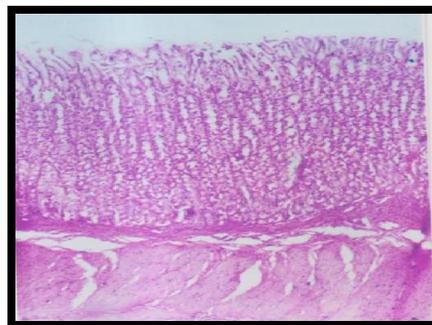


Fig. (5) Stomach of a rat given 20% *aloe vera* and *opuntia ficus* watery juice showing nearly normal histology of stomach. (H and E x 100)

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