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Gastro protective and Anti-Ulcer Activity of Aloevera and Amla Fruit Combined Juice in Pylorus Ligated Rats

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ABSTRACT

In the present study the anti ulcer activity of (1) *Aloe vera* juice, (2) *Aloe vera* and Amla fruit combined juice were investigated in the Pylorus Ligated induced ulcerated rats. The efficacy of both of the plant based juices were compared with the standard reference antiulcer drug pantoprazole. All the studies were conducted according to the ethical guidelines of CPCSEA. Healthy adult albino strains of Wister rats, weighing 150-200 g were used as experimental animals. Rats were divided into five groups comprising six rats in each group. Group I: Healthy animals received vehicle only (saline 2 ml). Group II: Disease control animals, ulcer was induced pylorus ligation. Group III: Ulcer induced rats treated with the *Aloe vera* juice (20.0 ml/kg-bw). Group IV : Ulcer induced rats treated with the *Aloe vera* and amla fruit combined juice(20.0 ml/kg-bw). Group V: ulcerated animals treated with pantoprazole(40mg/kg-bw). The administration of plant juices decreased the offensive factors like ulcer index, Gastric juice volume and pH, Total acidity, Acid volume and also reduced the amount of protein and carbohydrates in the stomach fluid. Further, plant juices increased the defensive glutathione. Activity factors like activity of oxidative enzymes such as superoxide dismutase and reduced of alkaline phosphatase and lipid peroxidase was higher in the diseased condition and same were reduced after the treatment with plant juices. The efficacy of plant juices was comparable with the standard drug- Ranitidine. The results of the present study reveal that the plant juices are having efficiency in the gastro protective activity. It is recommended that the above said plant derived juices can be further studied for their anti ulcer efficacy in human subjects.

Keywords: *Aloe Vera* juice, *Aloe Vera* and Amla fruit combined juice, pantoprazole pylorus ligated rats, Peptic ulcer, Antiulcer activity

ARTICLE INFO

CONTENTS

1. Introduction	16
2. Materials and Methods	16
3. Results and Discussion	17
4. Conclusion	18
5. References	19

Article History: Received 19 April 2016, Accepted 25 May 2016, Available Online 29 June 2016

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PAPER-QR CODE

Citation: P. Sailaja, et al. Gastro protective and Anti-Ulcer Activity of Aloevera and Amla Fruit Combined Juice in Pylorus Ligated Rats. *W. J. Pharm. Biotech.*, 2016, 3(1): 15-20.

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1. Introduction

Peptic ulcer is a painful sores or ulcers in the lining of the stomach or first part of the small intestine, called the duodenum which impairs the quality of life and is associated with increased morbidity and mortality. Ulcers are caused as a result of imbalance between aggressive and defensive factors. The aggressive factors include acid, pepsin, free radicals, infectious agents like *Helicobacter pylori*, chemicals and to a lesser extent bile salts and pancreatic enzymes. While the defensive factors include the adherent mucin, bicarbonate, prostaglandins and mucosa blood flow. [1]. The etiological factors are: diet [2], tobacco smoking [3], alcohol consumption [4], non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, naproxen and ibuprofen[5], corticosteroids[6], psychological stress [7], *H. pylori* infection [8] and genetic factors [9]. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Hence, efforts are on to find a suitable treatment from natural product sources. Reduction of gastric acid production as well as re-enforcement of gastric mucosal production has been the major approaches to cure peptic ulcer disease. As a result, more and more synthetic drugs are introduced and offering newer options for treatment of peptic ulcer.

The types of drugs vary from proton-pump inhibitor to H₂ antagonist or a cytoprotective agent. At the same time, each of these drugs confers simple or several side effects like arrhythmias, impotence, and gynaecomastia, hyperplasia and haemopoetic changes. Because of several side effects of synthetic medicines, there is new thought of better natural alternative for the treatment of peptic ulcer; constituents available in the medicinal plants have proved to be clinically effective and relatively less toxic than the existing synthetic drugs and reducing the offensive factors and serving as tool in the prevention of peptic ulcers. Several herbal plants are reported to have antiulcer activity and several pre clinical (animal) studies are reported on the efficacy of herbal medicines such as *Garcinia cambogia* [10], *Cissus quadrangularis* Linn. [11], *Tephrosia populnea* [12], *Bambusa arundinacea* [13], *Ocimum sanctum* [14], *Emblica officinalis* [15], *Pterospermum acerifolium* [16], *Bauhinia variegata* [17], *Terminalia chebula* [18], *Spheranthus indicus* [19], polyherbal extract containing *Curcuma longa*, *Coriander sativum* and *Ocimum sanctum* [20] and Plant juices such as *Aloe vera*, banana stem juice and banana flower juice [21] and *Carica papaya* (papaya) fruit juice [22]. The present study evaluates the antiulcer and gastro protective efficacy of (i) *Aloe vera* (*Aloe barbadensis* Mill.) leaf juice and (ii) combined juice of *Aloe vera* juice + Amla fruit juice in pylorus ligated induced ulcerated rats.

2. Material and Methods

Collection and preparation of plant juices:

Fresh leaves of aloe vera and amla fruit were collected from the Nellore, A.P. the plant materials were identified and World Journal of Pharmacy and Biotechnology

brought to laboratory. The tip and basal portions of aloe vera leaves were trimmed off and washed in clean water to remove soil and other dirty materials. Finally the leaves were soaked in clean sanitized water (containing 0.1% gramicid). After removing the rinds from the leaves, the inner gel was collected. The amla fruit was cleaned in sanitized water and cut into small pieces and again thoroughly washed. They were ground in a mechanical mixer to get the juices and they filtered through muslin cloth. The juices were stored in air tight container and kept 40c until further use. [23]

Animals

Healthy adult male albino rats of wistar strain weighing between 150-200g are used. They were housed in polypropylene cages and maintained under standard conditions (12 hrs light: 12 hrs dark cycle; 25±3°C and 35-60% humidity). The animals had free access to standard lab chow and tap water. All the experimental procedures were reviewed and approved by Institutional Animal Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals.^[24]

Experimental Procedure

Experimental design

The animals were divided into 5 groups each group contains 6 animals and were treated as shown below

Group I: Serve as control and will be received vehicle only (saline 2 ml).

Group II: Serve as disease control animals, ulcer was induced pylorus ligation

Group III: Ulcer induced rats treated with the Aloe vera juice (20.0 ml/kg-bw)

Group IV: Ulcer induced rats treated with the Aloe vera and amla fruit combined juice (20.0 ml/kg-bw)

Group V: Ulcerated animals treated with pantoprazole. Pantoprazole or its vehicle (CMC) (40mg/kg-bw)

Toxicity studies:

The toxicity studies of the ethonolic leaf and fruit extract were carried out in albino Rats of either sex weighing between 100-200gm. The LD₅₀ of ethonolic leaf extract was found to be safe till 2000mg/kg (i.p and p.o).

Induction of ulcer:

Pylorus Ligation Induced Ulcer Formation:

Albino rats of either sex were divided into four groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water. Animals in the control group received only distilled water. Methanol extract of *Aloe vera* and *Amla* at 20 ml/kg, (p.o) were given to the animals in the treatment group. Pantoprazole (40 mg/kg) was used as a standard. After 1h of drugs treatment, they were anaesthetized with the help of anesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay et al. [21], avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats

were sacrificed by an over dose of anaesthetic ether after four hours of pyloric ligation.[25] The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acidity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity [26].

Biochemical Parameters

The stomach was carefully excised keeping oesophagus closed and opened along greater curvature and luminal contents were removed. The gastric contents were collected in a test tube and centrifuged. The gastric contents were analyzed for gastric juice volume, pH.

Measurement of gastric juice volume and pH

Gastric juice was collected from pylorus ligated rats. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100g body weight. The pH of the supernatant was measured using digital pH meter. [27]

Ulcer index (UI) [28]

The mucosa was flushed with saline and stomach was pinned on dissecting board. The lesion in glandular portion was examined under a 10x magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Ulcer index of each animal was calculated by adding the values and their mean values were determined.

0 - colour- Normal d stomach

0.5 – Red colouration

1 – Spot ulceration

1.5 – Haemorrhagic streak

2 – Ulcers

3 – Perforations

Calculation of ulcer Index:

$$UI = UN + US + UP \times 10^{-1}$$

UI = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

Percentage inhibition

Percentage inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{UI_{\text{ulcer control}} - UI_{\text{ulcer treated}}}{UI_{\text{control}}} \times 100$$

Determination of free acidity and total acidity

Gastric juice specimen was transferred in a porcelain evaporating dish. 1-2 drops of methyl orange reagent is added. a bright red colour appears if free HCl is present. Titrated with 0.1 N NaOH from a burette, mixing was done after each addition until the last trace of red colour disappeared and was replaced by a canary yellow colour. The numbers of milliliters of NaOH used was read from the burette. This represents the amount of free hydrochloric acid (Y1). 1-2 drops of phenolphthalein were added to the gastric juice. The titration was continued until the red colour of phenolphthalein appeared (deep pink), titrated to the point at which the further addition of alkali

did not deepen the colour (Y2). Reading was taken (ml NaOH) for total acidity.[29]

Calculation

$$Y = Y_1 \times 10$$

Where,

Y = Free acidity (m Eq/L)

Y₁ = ml of NaOH consumed

$$Y = Y_2 \times 10$$

Where,

Y = Total acidity (m Eq/L)

Y₂ = ml of NaOH consumed

Acid volume

The stomach was removed and the contents were drained into a graduated centrifuge tubes through a small nick along the greater curvature adjacent to pyloric ligation. The volume of the juice was measured^[30]

pH:

The contents were drained into a graduated centrifuge tube. The tubes were centrifuged at 3000 rpm for 10 minutes and the centrifuged samples were decanted and analysed for pH (using broad range pH paper).^[31]

Statistical Analysis

All the values are expressed as mean ± S.E.M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison test. The values are statistically significant at three levels, ***p<0.001. **p<0.01. *p<0.05. But non significant if p > 0.05.

3. Results and discussion

Anti-Ulcer Screening

Pylorus ligation induced ulcer in Rats

Effects of aloe vera and amla combined juice on ulcer index by using pylorus ligation induced ulcer method in rats are shown in Table. Pylorus ligation induced gastric damage showed gross mucosal lesion, including long haemorrhage bands and petechial lesion. Animals pretreated with aloe vera and amla combined juice and standard drug pantoprazole showed very mild lesions and sometimes no lesion at all, when compared to ulcer control group.[32] Aloe vera and amla combined juice showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 26.53 and 53.06 at dose 40mg/kg doses respectively.

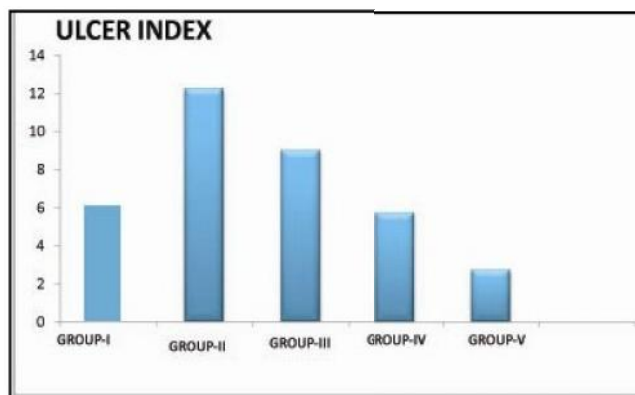


Figure 1: Effect of aloe vera and amla fruit combined juice on Ulcer Index in pylorus ligated rats.

The ulcer protective action of extracts at different doses was better as that of standard drug, pantoprazole, which exhibited an inhibition percentage of 77.5. Pylorus ligated rats showed severe gastric haemorrhagic lesions. (Mozafar khazaei *et.al.*, 2006) The pathogenesis of pylorus ligation induced gastric damage in rats is complicated and involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leucotriene C4. These mediators act on gastric microvasculature, triggering a series of events that lead to mucosal and sub mucosal damage. (Oates *et.al.*, 1988) So the cytoprotective mechanism of the aloe vera and amla combined juice may therefore include mechanisms other than simple acid neutralization. [33]



1. Normal Control



2. Ulcer Control



3. Ulcer treated with the Aloe vera juice (20ml/kg)



4. Ulcer treated with the Aloe vera



5. Pantoprazole (40mg/kg)

Figure 2: Effect of Aloe vera and amla fruit combined juice on pylorus ligation induced ulcer in rats.

Ulcer index (UI) and acid parameters:

The effects of aloe vera and amla fruit combined juice on acid parameters showed significant effect at 40mg/kg dose compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased and pH of the gastric juice was increased compared to ulcer control group. But, in this gastric environment also able to induce ulcer, so it can be thought that the antisecretory activity might not be the main mechanism of action of these extracts.[34]

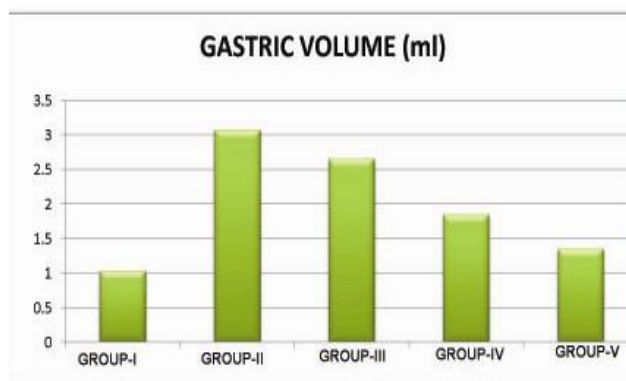


Figure 3: Effect of aloe vera and amla fruit combined juice on gastric secretion in pylorus ligated rats

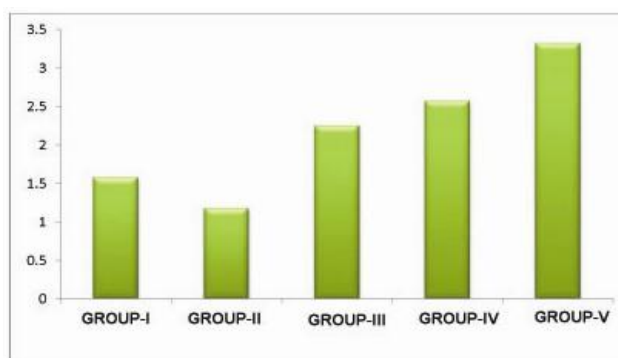


Figure 4: Effect of aloe vera and amla fruit combined juice on P^H in pylorus ligated rats.

4. Conclusion

The present study was undertaken to determine the gastroprotective and antiulcer activity of aloe vera and amla fruit combined juice in pylorus ligated in rats. Similarly, the ethanol extract of aloe vera and amla fruit combined juice

showed the presence flavonoids and their glycosides, tannins and triterpenoids. These phytoconstituents present in the juices could be the possible agents involved in the prevention of gastric lesions induced by pylorus ligation. Aloe vera and amla fruit combined juice showed a dose dependent curative ratio compared to ulcer control groups.

The extracts exhibited an inhibition percentage of 26.50 and 53.06 at doses of 200 and 400mg/kg doses respectively. The ulcer protective action of extracts at 40mg/kg was good to that of standard drug, pantoprazole, which exhibited an inhibition percentage of 77.50. [35]

Table 1: Effect of aloe vera and amla fruit combined juice on Ulcer Index in pylorus ligated rats

Group	Ulcer index (UI)	Percentage inhibition (%)
Normal Control	00.00 ± 0.00	-
Ulcer Control	12.25 ± 0.95	-
Ulcer treated with the Aloe vera juice (20ml/kg) Oral	9 ± 0.81**	26.53
Ulcer treated with the Aloe vera juice and amla fruit juice (20ml/kg) Oral.	5.25 ± 0.95***	53.06
Pantoprazole (40mg/kg) oral	2.75 ± 0.50***	77.5

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. Significant as compared to control $P^{***} < 0.001$, $P^{**} < 0.05$.

Table 2: Effect of aloe vera and amla fruit combined juice on Gastric secretion, pH in pylorus ligated rats

Group	Gastric volume (ml/100g)	pH of gastric juice
Normal Control	1.025±0.29	1.575±0.22
Ulcer control	3.075 ± 0.206	1.175±0.095
Ulcer treated with the Aloe vera juice (20ml/kg) Oral	2.65±0.208***	2.25±0.129**
Ulcer treated with the Aloe vera juice and amla fruit juice (20ml/kg) Oral.	1.85±0.129***	2.575 ±0.125***
Pantoprazole (40mg/kg) oral	1.35 ± 0.2***	3.32 ± 0.309***

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. Significant as compared to control $P^{***} < 0.001$, $P^{**} < 0.05$.

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