

Original Article

## ANTI-ULCER ACTIVITY OF ALOE VERA JUICE AND ALOE VERA AND AMLA FRUIT COMBINED JUICE IN ETHANOL INDUCED ULCERATED RATS

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### ABSTRACT

**Objective:** 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 ethanol induced ulcerated rats. The efficacy of both of the plant based juices were compared with the standard reference antiulcer drug Ranitidine.

**Methods:** 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 control animals; Group II: Disease control animals, ulcer was induced with 10.0 ml/kg-bw of 40% alcohol (ethanol); 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* + Amla fruit combined juice (20.0 ml/kg-bw); and Group V: Drug control animals- alcohol induced ulcerated animals treated with Ranitidine (50mg/kg-bw).

**Results:** The administration of plant juices decreased the offensive factors like ulcer index and acid secretion and also reduced the amount of protein and carbohydrates in the stomach fluid. Further, plant juices increased the defensive factors like activity of oxidative enzymes such as superoxide dismutase and reduced glutathione. Activity of alkaline phosphatase and lipid peroxidase was higher in the diseased condition and same were reduced after the treatment with plant juices. Content of haemoglobin and RBC and WBC counts were brought back to normalcy after the treatment with plant juices. The efficacy of plant juices was comparable with the standard drug- Ranitidine.

**Conclusion:** The results of the present study reveals that the plant juices are having efficiency in the gastroprotective 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, Amla fruit juice, Ranitidine, Peptic ulcer, Animal study, Antiulcer activity.

### INTRODUCTION

Peptic ulcer disease refers to 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. Peptic ulcer disease is a worldwide problem. Statistics from all sources indicate 10% or more of adult population are affected within their life time and peptic ulcer affects individuals from 20 to 60 years of age with males being predominantly affected. Considering the etiology of peptic ulcers, there are two prime factors responsible: the "aggressive" factors and the "defensive" factors. 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. Due to any cause, an increase in aggressive factors or a decrease in defensive factors will lead to loss of mucosal integrity resulting in ulceration[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. Currently there is no cost-effective treatment that meets all these goals. 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 H<sub>2</sub> 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.

Alternative approach in recent days is the research of medicaments from Ayurvedic and traditional medicinal systems. The phyto-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 alcohol induced ulcerated rats. Ranitidine is used as standard reference drug.

### MATERIALS AND METHODS

#### Collection and preparation of plant juices

Fresh leaves of *Aloe vera* and Amla fruit were collected from the Sri Padhuga Agriculture Farm in Samayapuram village, Tiruchirappalli District, Tamil Nadu State. The plant materials were identified and brought to laboratory. The tip and basal portions of *Aloe vera* leaves are 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 and ground. 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 4°C until further use.

### Experimental animals

Healthy adult albino strains of Wister rats, weighing 150-200 g were used as experimental animals. Animals were housed in polypropylene cages at 24±2°C in the college animal house and fed with commercial pellet diet and water *ad libitum*. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the institutional ethical committee (approval No: 790/03/ac/CPCSEA). The rats were divided into five groups comprising of six rats in each group.

### Experimental design

**Group I:** Healthy control animals

**Group II:** Disease control animals, ulcer was induced with 10.0 ml/kg-bw of 40% alcohol (ethanol).

**Group III:** Ulcer induced rats treated with the *Aloe vera* juice (20.0 ml/kg-bw) for 21 days.

**Group IV:** Ulcer induced rats treated with the *Aloe vera* + Amla fruit combined juice (20.0 ml/kg-bw) for 21 days.

**Group V:** Drug control animals- alcohol induced ulcerated animals treated with Ranitidine (50mg/kg-bw) for 21 days.

### Induction of ulcer

Animals were starved for 12 hours with access only to drinking water *ad libitum*. Gastric ulcer was induced with 10.0 ml/kg-bw of 40% alcohol (ethanol) induced in group II, III, IV and V animals.

After 48 hours an animal in Group II was sacrificed and checked for ulcer induction. Subsequently from the same day, Group III Animals were given *Aloe vera* juice 20.0 ml/kg- body weight (bw); Group IV animals were given *Aloe vera* juice + Amla fruit juice 20.0 ml/kg-bw. and Group V animals were given 50 mg/kg-bw of Ranitidine. Ranitidine was dissolved in distilled water and given to the respective animals. On 22<sup>nd</sup> day the animals were sacrificed. The animals were anaesthetized using ether. The blood samples were collected for haematological study. The abdomen was opened without causing any damage to its blood supply and an incision of 1cm long was made in the abdomen just below the sternum of the stomach was exposed. Passed a thread around the pyloric sphincter and applied a tight knot closed the abdomen wall by cervical decapitation and the stomach was removed. The gastric fluid was collected in a graduated centrifuge tube and subject to analysis for total acid, protein and carbohydrate contents. Samples of stomach tissues were collected and stored for enzymological analysis.

### Determination of ulcer index in the stomach [22,23]

In the cut opened stomach, the mucosa was washed slowly with saline and the stomach was pinned on frog board and observed under dissection microscope (10×) for ulcer mean score for each animal. The result is expressed as ulcer index by using following formula.

$$\% \text{ Ulcer index} = (\text{USc} - \text{Ust}) \times 100 / \text{USc}$$

USc = Ulcer surface area in control and USt = Ulcer surface area in treated animals.

### Determination of total acidity in gastric fluid [24]

The gastric fluid was centrifuged at 1000 rpm for 10 min, the volume was noted and pipette out 1ml of supernatant liquid. Diluted it to 10 ml with distilled water and titrated the solution against 0.01N sodium hydroxide (NaOH) using Topfer's -(Dimethyl-amino-azobenzene with phenolphthalein) reagent as indicator. Titrated up to the endpoint when solution turned to yellowish orange colour. Noted the volume of NaOH required for neutralizing the free hydrochloric acid present in the gastric juice. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. The difference between the two readings indicated the volume of NaOH required for neutralizing the

combined acid present in the gastric juice. The sum of the two titrations was the total acid present in the gastric juice.

Acidity was calculated as follow:

$$\text{Acidity} = \text{Vol. of NaOH} \times \text{Normality of NaOH} / \text{Vol. of gastric juice used}$$

The total acidity was expressed as mEq/dl

### Estimation of total protein (Modified method of Lowry *et al.* [25,22])

The content of dissolved total protein in the gastric fluid was estimated in the alcoholic precipitate obtained by adding 90% of alcohol with gastric fluid in 9:1 ratio. Then 0.1 ml of alcoholic precipitate gastric fluid was dissolved in 1 ml of 0.1 N NaOH and from this 0.05 ml was taken in another test tube. To this 4 ml of alkaline copper reagent was added and kept for 10 min. Then 0.5 ml Folin's phenol reagent was added and kept for 10 min and the solution was allowed for colour development. Reading was taken against blank prepared with distilled water at 640 nm. The protein content was calculated from standard curve prepared with bovine albumin and has been expressed in terms of µg/ml of gastric juice. (Standard Bovine serum albumin: 20 mg of bovine serum albumin was dissolved in 100 ml distilled water. Few drops of NaOH were added to it to aid complete dissolution of bovine serum albumin and to avoid frothing and it was allowed to stand over night in a refrigerator).

### Estimation of carbohydrates (Modified Anthrone method [22])

To 0.1 ml of gastric fluid 2 ml of 3N sulphuric acid and 2 ml of sodium tungstate were added and mixed. The content was centrifuged at 3000 rpm for 10 min. From this 0.4 ml of supernatant was taken in a series of test tubes and the blank was prepared without gastric fluid. The volume in all test tubes was made up to 1 ml by adding distilled water. After 10 min of incubation in ice cold water bath, 4 ml of anthrone reagent (freshly prepared by dissolving 0.2 g of anthrone in 100 ml of concentrated sulphuric acid) was added in to all the test tubes. The test tubes were kept in the boiling water bath for 15 min. After cooling down the solution, read the optical density at 540 nm. The carbohydrate content was calculated from standard curve prepared with different strength standard glucose solution (10-100 µg of glucose dissolved in 1ml distilled water) and the results has been expressed in terms of µg/ml of gastric fluid.

### Assay of serum alkaline phosphatase [26]

The reaction mixture was prepared by adding 1.5 ml of 0.1M carbonate buffer (pH 10), 1 ml of 0.1M disodium phenyl phosphate and 0.1 ml of 0.1M magnesium chloride and finally 0.1 ml of gastric fluid was added. The reaction mixture was incubated at 37°C for 15 min and the reaction was arrested by the addition of Folin's phenol reagent. Control tubes were treated similarly but serum was added after the reaction was arrested by the Folin's phenol reagent. 1ml of 15% sodium carbonate was added and the colour developed was read after 10 min at 640 nm. The enzyme activity was expressed as IU/ml.

### Assay of reduced glutathione [27]

To 0.5 ml of tissue homogenate, 20% TCA was added and precipitated. The contents were mixed well for complete precipitation of protein and centrifuged. To aliquots of clear supernatant, 2.0 ml of DTNB reagent (0.6mM DTNB in 0.2 M Phosphate buffer, pH 8.0) was added and 0.2 M phosphate buffer was added to make a final volume of 4.0 ml. The absorbance was read at 412 nm against a blank containing TCA instead of sample. A series of standard solutions (prepared from 10 mg of reduced glutathione dissolved in 100 ml of water) were treated in a similar way to determine glutathione content. The amount of glutathione was expressed as nano moles of GSH oxidized/mg protein.

### Assay of lipid peroxidase [28]

To 0.1ml of tissue homogenate, 4 ml of 0.85N H<sub>2</sub>SO<sub>4</sub> and 0.5 ml of 10% phosphotungstic acid were added and stirred well. The content was centrifuged at 5000 rpm for 10 min. The supernatant was

discarded and the sediment was mixed with 2.0 ml of H<sub>2</sub>SO<sub>4</sub> and 0.3 ml of 10% phosphotungstic acid. The mixture was centrifuged for 10 min. The sediment was suspended in 4.0 ml of distilled water and 1 ml of TBA reagent (mixture of equal volumes of 0.67% TBA aqueous solution and glacial acetic acid). The tube was kept in a boiling water bath for 1 hr, after cooling, 5 ml of butanol was added and the colour of the extract in the butanol phase was read at 532 nm. The reaction mixture without tissue homogenate was used as blank. The enzyme activity was expressed as nano moles/mg tissue protein.

#### Activity of superoxide dismutase [29]

To 0.1ml of tissue homogenate, 0.75 ml of ethanol and 0.15 ml of ice cold chloroform were added and centrifuged. The supernatant was taken and 0.5 ml of 0.6nM EDTA solution and 1 ml of buffer (0.1 M pH 10.2) were added and mixed well. The reaction was initiated by the addition of 0.5 ml of fresh epinephrine (1.8nM) and the increase in absorbance was measured at 480 nm. The reaction mixture without tissue homogenate was used as blank. The enzyme activity was expressed as U/ml.

#### Studies on haematological parameters [22]

##### Determination of haemoglobin

A series of (6 Nos.) test tubes were taken and 0.02 ml of blood sample was added in to each test tube. Then 5ml of Drabkin's reagent was added and the test tubes were allowed to stand for 4 min. The blank was prepared similarly without adding blood sample and the read reaction solution at 540 nm in a calorimeter.

#### Enumeration of RBC count

The anticoagulated blood was sucked into pipette up to the marking of 0.5. It was diluted with RBC diluting fluid (Hayem's diluting fluid) by sucking up to 10 marking. The pipette was shaken for a min and wiped away the excess of blood by using blotting paper. Placed a drop of blood at the edge of haemocytometer and placed a cover slip on it, which covered the counting chamber. The cells were counted under microscope on the 16 squares in 5 different parts of the fluid.

#### Enumeration of WBC count

The anticoagulated blood was sucked into pipette up to the marking of 0.5. It was diluted with WBC diluting fluid (Turkey's fluid) by sucking up to 10 marking. The pipette was shaken for a min and wiped away the excess of blood by using blotting paper. Placed a drop of blood at the edge of the haemocytometer and placed a cover slip on it, which covered the counting chamber. The cells were then counted under microscope on the 4 corner squares.

#### Statistical analysis

The data of results obtained were subjected to statistical analysis and expressed as mean  $\pm$  SD. The data were statically analyzed by one way analysis of various (ANOVA) and to compare the means of the studied groups with *post hoc* Duncan multiple range tests at 5% and 1% for those results where significant difference was indicated. Minitab statistical software was used.



Fig. 1(a): Group I: Healthy control animal



Fig. 1(b): Group II: Ulcer induced animal



Fig. 1(c): Group III: Ulcer induced animal treated with Aloe vera juice



Fig. 1(d): Group IV: Ulcer induced animal treated with Aloe vera + Amla juice



Fig. 1(e): Group V Ulcer induced animal treated Treated with Ranitidine

Fig. 1: Photographs showing ulceration in the experimental animals

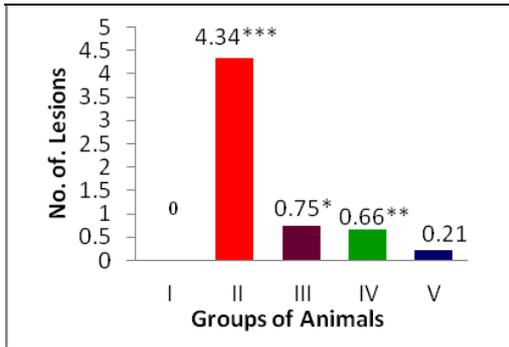


Fig. 1: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on ulcer index in alcohol induced ulcerogenic rats

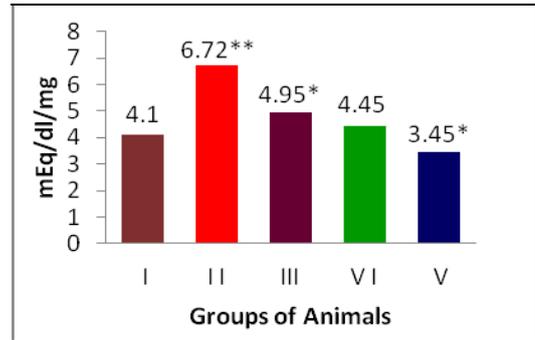


Fig. 2: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on Total Acidity in alcohol induced ulcerogenic rats.

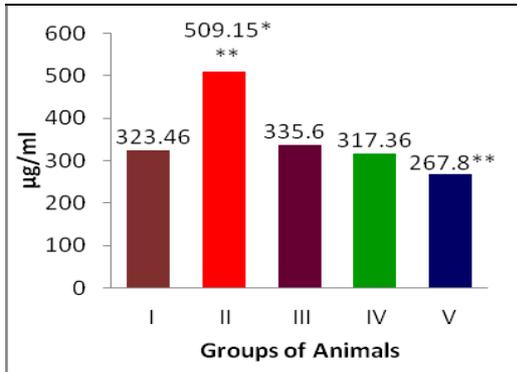


Fig. 3: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on of total protein levels in alcohol induced ulcerogenic rats

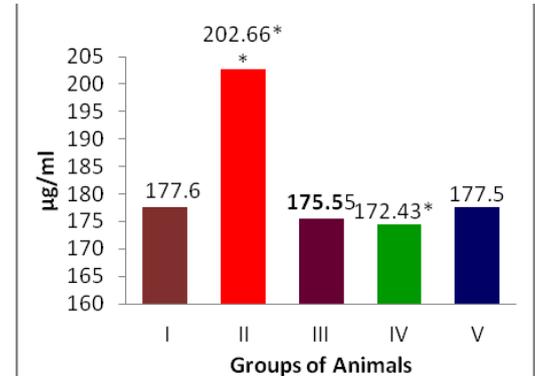


Fig. 4: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on carbohydrates levels in alcohol induced ulcerogenic rats.

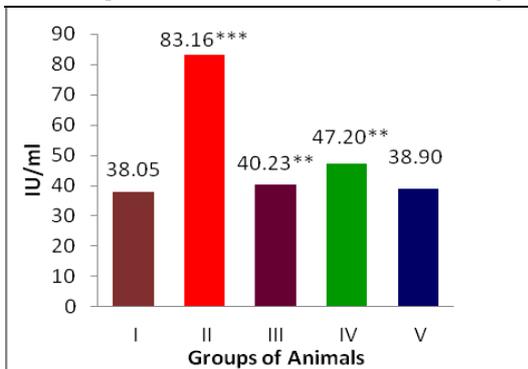


Fig. 5: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on Alkaline phosphatase activity in alcohol induced ulcerogenic rats

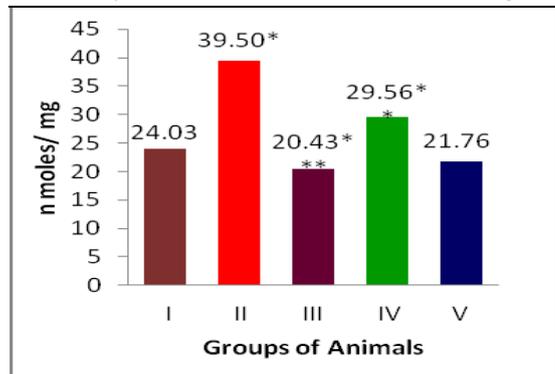


Fig. 6: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on Lipid peroxide in alcohol induced ulcerogenic rats

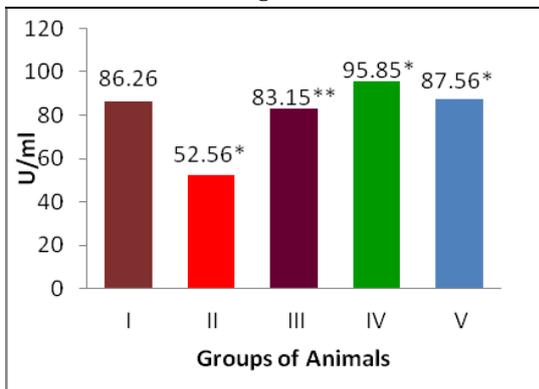


Fig. 7: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on Reduced glutathione activity in alcohol induced ulcerogenic rats

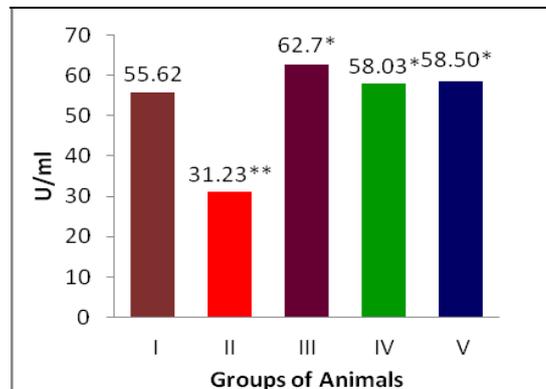


Fig. 8: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on Superoxide dismutase activity in alcohol induced ulcerogenic rats

Values are expressed as means ± S.E.M (n=6) \*P<0.05, \*\* P<0.01, \*\*\*P<0.001 compared with control group

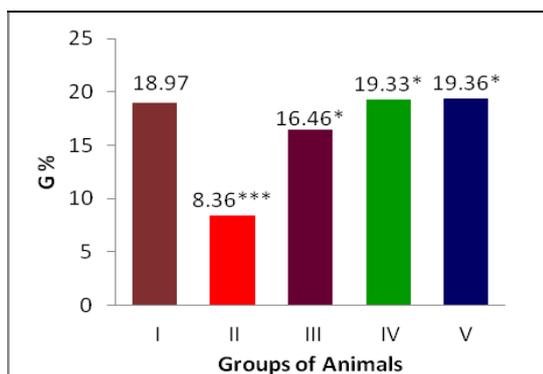


Fig. 9: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on Hemoglobin content in alcohol induced ulcerogenic rats.

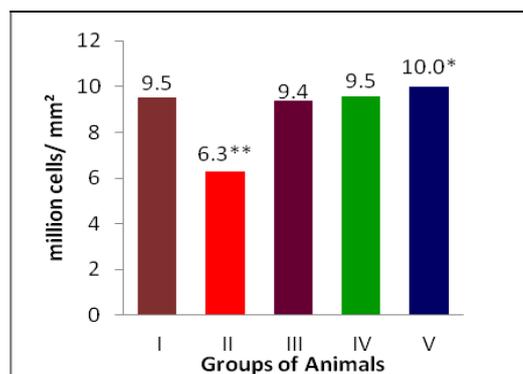


Fig. 10: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on Red Blood Cells count in alcohol induced ulcerogenic rats.

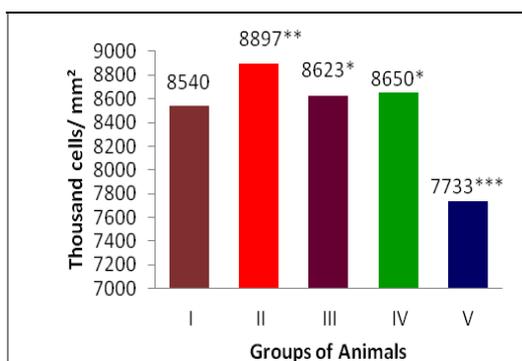


Fig. 11: Effect of *Aloe vera* juice and *Aloe vera* + Amla fruit combined juice on White Blood Cells (WBC) count in alcohol induced ulcerogenic rats.

Values are expressed as means  $\pm$  S.E.M (n=6) \*P<0.05, \*\* P<0.01, \*\*\*P<0.001 compared with control group

## RESULTS

The results on the effect of *Aloe vera* juice and combined juice of *Aloe vera* and Amla fruit combined juice reveals that the plant juices (plant drugs) have significantly ( $p < 0.001$ ) reduced the ulcer index (PLATE 1). The gross examination of the stomach in the gastric ulcer induced group of animals (Group I) showed marked indurations, dilated blood vessels, ecchymosis and haemorrhagic sites in the serosal surface. The mucosal surface also exhibited with features of severe degree of hyperemia, congestion and large number of pin point ulcers of varying sizes with central clots and feature for perforation in the stomach (Plate 1a & 1b) Ulcer index was severe ( $4.34 \pm 0.1$ ) in the disease control (ulcer induced animals) and the same was decreased in other groups of animals which were treated with plant juices. Maximum inhibition of ulcer index was observed in the animals treated with of *Aloe vera* + Amla fruit combined juice (Plate 1c, 1d). Animals treated with plant juices and Ranitidine showed healing symptoms of ulcer (Plate 1e). The serosal surface of the drug treated animals revealed very few dilated blood vessels and haemorrhages and mucosal surface revealed few ulcer markings. The ulcer index was also reduced to  $0.75 \pm 0.05$ ,  $0.66 \pm 0.03$ , and  $0.21 \pm 0.06$  in Group III, IV and V animals respectively.

Total acidity was increased in the ethanol induced ulcerated rats ( $6.72 \pm 3.1$  mEq/dl/mg) and the same was decreased in the ulcerated rats after the administration plant drugs  $4.95 \pm 4.6$ ,  $4.45 \pm 3.4$  and  $3.45 \pm 5.2$  in the group III, group IV, and group V respectively) (Fig. 2)

The level of total protein in the stomach juice was increased in the ulcer induced animals ( $509.15 \pm 8.2$   $\mu$ g/ml), when compared to healthy control animals ( $323.46 \pm 9.4$ ). But the intake of plant drugs significantly decreased the protein and levels ( $317.36 \pm 5.1$  and  $317.36 \pm 5.1$  in group III, and IV animals respectively). Administration of Ranitidine also reduced protein content in the stomach juice ( $267.80 \pm 5.52$ ) (Fig. 3). The ulcer induced animals showed higher content of carbohydrate ( $202.66 \pm 9.3$   $\mu$ g/ml) than the control

animals ( $177.60 \pm 6.2$ ). But the animals administrated with of natural juices and Ranitidine showed reduced carbohydrate contents ( $175.556 \pm 8.3$ ,  $174.43 \pm 6.2$  and  $177.50 \pm 7.20$  in Group III, IV, and V animals respectively) (Fig. 4)

The activities of antioxidant enzymes such as alkaline phosphatase, lipid peroxidase, increased in the ulcer induced animals and the treatment with plant drugs and Ranitidine decreased the activities (Fig.5). The activity of alkaline phosphatase was  $38.05 \pm 4.2$  IU/ml in control animals which was drastically increased to  $83.16 \pm 3.4$  in ulcer induced animals. The animal treated with plant drugs and Ranitidine exhibited lesser enzyme activity ( $40.23 \pm 3.5$ ,  $47.20 \pm 5.4$  and  $0.21 \pm 0.13$  in group III, IV, and V animals respectively) (Fig. 5).

The lipid peroxidase activity was  $24.03 \pm 2.1$  n moles/mg in the healthy animals. After ulcer induction peroxidase activity was increased to  $39.50 \pm 2.1$ . The administration of plant juices brought back the enzyme activity to  $20.43 \pm 3.2$  and  $29.56 \pm 6.2$  in Group III and IV animals respectively. The administration of Ranitidine also restored the enzyme activity ( $21.76 \pm 3.3$ ) (Fig. 6)

The activity of reduced glutathione was decreased in the ulcer induced group of animals ( $52.56 \pm 3.4$  U/ml) than the healthy control animals ( $86.26 \pm 4.3$ ). The activities of these enzymes were increased in the animals treated with plant drugs and Ranitidine ( $83.15 \pm 4.2$ ,  $95.85 \pm 5.5$ , and  $87.56 \pm 5.2$  in Group III, IV, V and VI animals respectively) (Fig. 7).

The activity of superoxide dismutase (SOD) was drastically reduced in the ulcer induced animals ( $31.23 \pm 3.4$  U/ml) than the healthy animals ( $55.62 \pm 3.5$ ). The administration of plant juices and Ranitidine enhanced the SOD activity ( $62.70 \pm 4.3$ ,  $58.03 \pm 4.2$ , and  $58.50 \pm 7.6$  in Group III, IV and V respectively) (Fig. 8).

The haemoglobin level was  $18.97 \pm 2.5\%$  in healthy animals and it was drastically decreased to  $8.36 \pm 1.1\%$  in the ulceration condition. But the haemoglobin content was significantly ( $p < 0.01$ ) improved

the in animals which were treated with plant drugs and standard drug (16.46±4.2%, 19.33±5.4%, and 19.36±1.9% respectively in Group III, IV and V animals respectively. Further the effect was comparable with healthy control animals (Fig. 9).

The count of Red Blood Cells (RBC) was 9.50±0.8 million cells/mm<sup>2</sup> in healthy control animals. Ulcer induction significantly ( $p < 0.05$ ) reduced the count (6.30±0.4). The treatment with plant juices and standard drug significantly enhanced the RBC count (9.40±0.8, 9.55±0.4 and 10.00±0.4 in Group III, IV and V animals respectively) (Fig. 10).

The White Blood Cells (WBC) count was higher in the disease control Group II rats (8897 ±15.2 thousand cells/mm<sup>2</sup>) than healthy control, Group I animals (8540±16.4). But the administration of plant drugs brought down the WBC count in plant drug treated animals (8623±12.5, 8650±15.5 and in III, and IV Group animals respectively. The animals treated with ranitidine the WBC count was lesser (7733±20.6- Group V) than healthy animals (Fig. 11).

## DISCUSSION

The experimental animals were divided into 6 groups and each group consisting of 6 animals.

Animals in group I were maintained as healthy control where as animals in the group II, III, IV and V were administered with 40% ethanol and ulcer was induced. An animal in the group II was sacrificed after 48 hrs of ulcer induction and checked the stomach for ulceration. Ulcer was noticed and the observation confirmed that the group II, III, IV and V animals were ulcerative. The plant drugs, such as *Aloe vera* juice and combined juice of *Aloe vera* juice and Amla fruit and standard drug Ranitidine were started administered from the same day. On 22<sup>nd</sup> day after treatment the animals were sacrificed and analyzed for the curative effect of tested drugs.

The administration of alcohol has induced the peptic ulcer in the experimental animals and the ulcerated condition increased the levels of offensive factors such as ulcer index and total acidity. Administration of plant drugs significantly decreased the levels of offensive factors.

Total protein and carbohydrate levels in the stomach fluid were increased during ulcerogenesis. It is reported that ethanol has the ability to damage the gastric mucosa by mechanical injury [30] and ethanol induced gastric lesion formation may be due to stress in the gastric blood flow which contributes to the development of hemorrhage and tissue damage due to necrosis [22]. Further, alcohol combined with hydrochloric acid present in the gastric juice induces numerous punctiform and filiform gastric ulcers during the course of necrosis and increase the tissue damage. Because of the tissue damage, the levels of protein and carbohydrates were increased in the stomach fluid of ulcerated animals. Administration of plant drugs has decreased the levels of protein and carbohydrates in the animals. This indicates that plant drugs healed the ulcer wounds in the stomach, so that disintegration of protein and carbohydrate from tissues was prevented and the dissolution of protein and carbohydrates in the stomach fluid was reduced. The treatment with *Aloe vera* juice and Amla fruit juices may enhance the gastric mucosal defensive factors. Therefore the protection afforded by plant juices against ethanol induced gastric ulceration may be due to inhibition of the 5-lipoxygenase pathway or to the antagonistic activity of leukotrienes. Flavonoids are implicated in the protection of the gastric mucosa from necrotizing substances [31] and flavonoids are highly useful in the therapy of acute and chronic gastric ulceration [31], [22].

Habib-ur-Rehman [32] reported the presence of flavonoids such as kaempferol 3-O-alpha-L-(6''-methyl) rhamnopyranoside and kaempferol 3-O-alpha-L-(6''-ethyl) rhamnopyranoside in the fresh Amla fruit. Khan [33] reported that quercetin is the major flavonoid present in the Amla fresh fruit. Flavonoids have been reported as anti-ulcer agents [34], [35], [36]. The antiulcer and gastroprotective effects of quercetin and its glucosides are reported by many researchers. Martin *et al.* [37] and Kahraman *et al.* [38] reported the antioxidant mechanisms involved in gastroprotective effects of flavonoids in ethanol induced ulcerative rats. Antiulcer activity of flavonoids by stimulating Platelet Activator Factor (PAF) in acid-

ethanol induced ulcerative animal model was reported by Izzo *et al.*, [39]. Effects of quercetin and other flavonoids on the reserpine induced ulcerogenic mice is documented by Barnaulov *et al.* [40]. Motilva *et al.* [41] recorded the effects of naringenin and quercetin on the acetic acid induced ulcerogenic rats.

Alkaline phosphatase and lipid peroxidase were found higher in the ulcer induced animals. The release of more amount of alkaline phosphatase has been suggested to play a role in the tissue necrosis process and the increasing trend of alkaline phosphatase was observed with various other animal models of gastrointestinal ulceration [42]. In the present study the activities of alkaline phosphatase, and lipid peroxidase were higher in the diseased condition and the same were reduced after the treatment with plant drugs. Further the activities were comparable with healthy control animals, which reveal that the plant drugs brought normalcy in the activities of these enzymes.

Reduced glutathione and superoxide dismutase are considered as antioxidant enzymes which are responsible for the antioxidant activities (scavenging and disposal of free radicals from the tissues). In the present study activities of reduced glutathione and superoxide dismutase enzymes were decreased in the ulcerated animals, where as the activities of these enzymes were increased in the animals which were treated with plant drugs. Further, the animals treated with standard drug (ranitidine) showed lesser enzyme activity than the animals treated with plant drugs. This observation clearly indicates that the plant drugs are more capable of enhancing the antioxidant activity. This action may be due to the availability of antioxidant phytochemicals in the plant drugs. Several phenolic compounds were isolated to present in fresh fruit of Amla such as gallic acid, ellagic acid, 1-O-galloyl-beta-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulinic acid, quercetin, chebulagic acid, corilagin, 1,6-di-O-galloyl beta D glucose, 3-Ethylgallic acid (3-ethoxy 4,5-dihydroxy benzoic acid) and isostrictin [43]. Further, Bhattacharya *et al.* [44] reported that the potent vitamin C like activity of Amla fruit was due to low molecular weight (Mw <1000) hydrolysable tannins. Four such compounds, emblicanin A, emblicanin B, punicalunin and pedunculagin were isolated from the pericarp of fresh Amla fruit. Emblicanin A and B shown to exhibit significant antioxidant effect *in vitro*. These phenolic compounds may be responsible for the enhanced antioxidant activity of Amla fruit juice. There are evidences that consumption of antioxidant phenolic compounds is associated with prevention chronic diseases such as cancer, diabetes and cardiovascular disease. The present study proves that phenolic compounds are beneficial in gastroprotection and antiulcer activity.

It is reported that Amla fruit juice contains quercetin and other flavonoids and *Aloe vera* juice contains glucomannans, amino acids, lipids, sterols and vitamins. Roa *et al.* [45] reported that bioflavonoid protects against oxidative stress related to ulcers in rats. Further, Russo *et al.* [46] reported that flavonoids act as antiradical and antioxidant agents and protect cell/tissue damage.

Anti-inflammatory action of *Aloe vera* gel is well documented. *Aloe vera* gel inhibits the cyclooxygenase pathway and reduces prostaglandin E<sub>2</sub> production from arachidonic acid. Recently, the novel anti-inflammatory compound called C-glucosyl chromone was isolated from *Aloe vera* gel extracts. This may be the possible explanation for ulcer curative property of *Aloe vera* juice [47].

The hematological parameters such as haemoglobin, RBC, and WBC are indicators of healthy physiological function of the circulatory system. It is well documented that biosynthesis of haemoglobin and RBC were decreased during ulcer disease condition. The same observation was made in the present study also. Both haemoglobin and RBC contents were lesser in the ulcerated rats when compared to the healthy rats. This may be due to the breakdown of haemoglobin and RBC in the ulcerated condition [22]. Treatment with combined juice of *Aloe vera* and Amla fruit enhanced the haemoglobin content and RBC counts. Gopinathan [21] reported that reduction in the haemoglobin contents and RBC counts were increased by the treatment with *Aloe vera* juice and *Aloe vera* juice in combination with banana stem juice and banana flower juice in the alcohol induced ulcerated rats.

Ulcerogenesis drastically enhanced the production of white blood cells (WBC) in the rats. This may be due to the over production of WBCs during disease condition to combat the pathogens which were infected in the ulcerated tissues. Further, it was observed that WBCs count was brought to normal after the treatment with plant drugs and count was comparable to healthy control animals.

From the present study, it is well documented that all the plant drugs such as *Aloe vera* juice has the efficacy in curing peptic ulcer, which is shown in their ulcer defensive factors. But *Aloe vera* juice in combination with Amla fruit juice showed better performance in all parameters studied. This may be attributable because of the phyto-nutrients and phyto-chemicals present the combined juice.

The anti ulcer activity of *A. vera* is due to its anti-inflammatory [48], cytoprotective [49], wound healing [50], antioxidant and mucus stimulatory effects [22] and the gastroprotective and anti ulcerogenic effects of Amla juice may be due its phyto-constituents like flavonoids and tannins. Rebecca et al. [51] reported that tannins extracted from *Syzygium cumini* had showed a significant decrease in gastric mucosal damage and reduce ulcer lesions.

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