EFFECT OF
PARTIALLY PURIFIED
FRACTIONS FROM
ALOE VERA ON
CHEMICALLY -INDUCED
ULCERS IN
COLON, STOMACH
AND MOUTH
5. EFFECT OF PARTIALLY PURIFIED FRACTIONS FROM ALOE VERA ON CHEMICALLY – INDUCED ULCERS IN COLON, STOMACH AND MOUTH

5.1 INTRODUCTION

Column chromatography is generally used as a purification technique; it isolates desired compounds from a mixture. It includes, the stationary phase, a solid adsorbent (silica gel and alumina), placed in a vertical glass (usually) column and the mobile phase, a liquid, added to the top and flows down through the column either by gravity or external pressure. The adsorbents viz. silica gel will be in different mesh size eg. 60, 60-120, 70-230 etc. The number refers to the mesh of the sieve used to size the silica, specifically, the number of holes in the mesh or sieve through which the crude silica particle mixture is passed. If there are more holes per unit area, those holes are smaller, thus allowing only smaller silica particles go through the sieve. The relationship is; the larger the mesh size, the smaller the adsorbent particles.

Ulcerative colitis is a chronic inflammatory disease of the colon and rectum. While the exact cause remains unknown, possible etiological factors, including genetic, immunologic and environmental have been implicated (Jewell and Patel, 1985). As the exact etiology and pathogenesis of the disease development is unclear, the treatment of ulcerative colitis becomes major obstacle. Commonly used medications viz. aminosalicylates, glucocorticoids, antibiotics and immunomodulators (Shanahan, 2001) offer temporarily relief for ulcerative colitis. As the current treatment is not much satisfactory to treat the disease, polyherbal formulations have been studied in ulcerative colitis due to their antioxidant and anti-inflammatory action (Harputluoglu et al., 2006). Thus, the use of medicinal plants or their active components have become an increasingly attractive approach for the treatment of ulcerative colitis. In a recent work, the alcoholic extract of Aloe vera was found to reduce chemically induced oxidative stress in rats (Anilakumar et al., 2010). Keeping in view of the potent anti-inflammatory activity of Aloe vera observed in experimental studies, this study was undertaken to investigate the anti-ulcerative effect of antioxidant rich fraction of Aloe vera gel.
Gastric ulcers are among the most important diseases in the world. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products and drugs (Peskar and Mericic, 1998). These agents have been implicated in the pathogenesis of gastric ulcer viz. an increase in gastric acid and pepsin secretion, a decrease in gastric blood flow, the suppression of endogenous generation of prostaglandins, inhibition of mucosal growth and cell proliferation and alteration of gastric mobility (Kontarek et al., 1998). Most of the population in developing countries still relies on traditional medicine practitioners and local medicinal plants for primary health care. As an alternative to other approaches (Garcia et al., 1978; Rimbau et al., 1999), scientific research on plants used in traditional medicine in receiving growing interest as a way of identifying new agents. Aloe vera is a chemically ill-defined extract of the Aloe barbadensis miller plant. There is no doubt that this compound is bioactive (Logarto et al., 2001). Phytomedicine ascribes anti-inflammatory, analgetic, liver-protective, anti-proliferative, anti-carcinogenic, anti-aging, and laxative effects to this plant (Swanson, 1995; Fischer, 1982; Syed et al., 1996; Ikeno et al., 2002). These effects are thought to be the result of radical scavenging, inhibition of COX-2, and immunomodulatory mechanisms. The aim of this study was to corroborate the antiulcer effect attributed to Aloe vera leaf gel by evaluating the Aloe vera fraction.

Aphthous ulcers and/or aphthous stomatitis are used to describe oral mucosal wound. Oral mucosal wound or mouth ulcers are sores or open lesions in the mouth which are caused by various disorders (Mandell, 2000; Gonsalves et al., 2007). Recurrent oral ulcer is one of the most common oral mucosal diseases. Oral ulcer (or mouth ulcers) can be very painful and the resulting lesions can be mild or severe. Although oral ulcer has been the subject of considerable studies, the etiology and pathogenesis of the disease have not yet been completely explained (Tran et al., 1996). Local and systemic conditions, genetic, immunological and infectious factors have been identified as potential etiological agents. Recurrent oral ulcer is a chronic systemic inflammatory disease. Inflammatory reactions trigger the oxidative stress and oxidants decrease the level of antioxidants. The free radical metabolism in the erythrocyte and serum of oral ulcer patients has been investigated in two studies (Katayama et al., 2000; Orem et al., 2002). Aloe vera has been consumed as a traditional Chinese medicine in single and compounding prescriptions for treating
fever, constipation and ringworm. Some specially prepared *Aloe vera* extracts possess many biological activities such as anti-inflammation, anti-cancer, antioxidant, anti-diabetes and macrophage activation (Grover et al., 2002; Krishnan, 2006; Xiao et al., 2007; Xu et al., 2008).

### 5.2 MATERIALS AND METHODS

#### 5.2a MATERIALS

This is earlier provided in the Chapter No.3

#### 5.2b METHODS

#### 5.2b.1 Preparation of partially purified fraction of *Aloe vera*

The methanol extract of *Aloe vera* (~20 g) was subjected to column (450x40 mm) chromatography using silica gel (60-120 mesh). The column was loaded with methanol extract of *Aloe vera* (since extract was hygroscopic, it was mixed with silica gel in the ratio of 1:1. The mixture was dried at 40°C to remove moisture and it was finely powdered using a pestle and mortar). The fractions were eluted stepwise with different solvents (the eluent), using different ratios (Fig 39) and subsequently collected in time duration of 1 ml/min, which is passed through the column by the application of air pressure. Each fraction of 50 ml is collected and total of 450 ml of fraction from each respective solvent is collected. Further, the fractions were concentrated by vacuum and dissolved in known volume. Thin Layer Chromatography was performed in each concentrated and redissolved fractions using fluorescent silica gel TLC Aluminum sheets (20x20cm) to ascertain the presence of compounds. The plates were run in ascending direction from 18 to 19cm of height with different proportions of chloroform and methanol as mobile phase. After air-drying, the spots on the plates were located by illumination under UV chamber. The fractions were pooled based on the Retention Time as calculated from the TLC plates. Further, these pooled fractions were analyzed for antioxidant scavenging property by radical scavenging method.
Fig 39. Schematic representation of eluted solvents in different ratios

pet.ether (F1-9) | chloroform (F37-45) | ethylacetate (F73-81) | acetone (F110-118) | n-butanol (F138-146) | methanol (F174-182) | water (F210-218)
<table>
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<td>100</td>
</tr>
</tbody>
</table>

pet.ether: chloroform | ethylacetate: acetone | n-butanol: methanol: water
| pet.ether: chloroform | ethylacetate | acetone | n-butanol | methanol | water
| 90:10 (F10-18) | 50:50 (F19-27) | 10:90 (F28-36) | 90:10 (F183-191) | 50:50 (F192-200) | 10:90 (F201-209) |

Total 218 fractions
5.2b.2 Antioxidant activity by radical scavenging method

This is earlier provided in the Chapter No. 2

5.2b.3 Preparation of glucomannan an Aloe vera polysaccharide

*Aloe vera* gel is known to contain unstable compounds that include glucomannan. Therefore, an efficient and accurate method was used colorimetrically for the determination of glucomannan in *Aloe vera* products.

The polysaccharide standard was prepared by the method of Eberendu et al. (2005). The mucilaginous parenchyma tissue of the *Aloe vera* leaf was filleted, homogenized in a blender at maximum speed, and filtered through 8 layers of muslin cloth. The filtrate was freeze dried to an off-white solid. The dried solids were ground to a powder. About 5 g of crude powder was dissolved in 1 liter deionized water. The mixture was placed in an orbital shaker for 4 hrs. The solution was clarified by passing it through a 1.2 µm membrane syringe filter. It is then transferred into dialysis tubing of 8000 Da molecular weight cut off. After 48 hrs of continuous dialysis and 5 changes of water, the retained polysaccharide was again freeze dried and stored in a tight plastic container at room temperature. Freeze dried *Aloe vera* polysaccharide standard (20 mg) was weighed on an analytical balance and it is dissolved in 20 ml distilled water to a concentration (1 mg/ml) stock solution. Working standard solutions of 10, 20, 100, 500, and 800 µg/ml were prepared by diluting appropriate aliquots of stock solution with distilled water. To prepare calibration standards, aliquot samples (400 µl) of each concentration level were transferred into disposable glass culture tubes. To each tube was added 4 ml Congo red reagent and mild vortexing was used to mix. The mixture was left at room temperature for 20 min. Absorbance was then taken at 540 nm wavelength.

5.2b.4 Effect of Aloe vera fractions on cyclooxygenase -2 inhibitory activity

*In vitro* enzymatic activity of COX 1 and 2 was measured using chromogenic assay based on the oxidation of N,N,N,N-tetramethyl p-phenylene diamine (TMPD) during the reduction of prostaglandin G₂ to PGH₂ (Kulmacz and Lands, 1983;
Copeland et al., 1994). The assay mixture in 1 ml contained 3µl of 40nM of TMPD (final concentration 120 µM), 5µl of 20 mM arachidonic acid (final concentration 100 µM), 5-10 µM of enzyme (50-100 µg of protein and 987 µl of buffer (100 mM Tris pH 8.0; 10 µM Hematin and 3 µM EDTA). The enzymatic activity was measured by estimation of the initial velocity of TMPD oxidation in the reaction as increase in absorbance at 603 nm. The COX activity was expressed in units/ml enzyme or mg protein. One unit of enzyme is defined as the amount of enzyme required to oxidize one nanomole of TMPD per min at 25° C. Of the 7 fractions, petroleum ether, chloroform, ethyl acetate, n-butanol and water fractions showed inhibition activity by COX assay. Fractions showing COX inhibitory activity was further used to study in vivo anti-inflammatory activity in albino rats of Wistar strain using the carrageenan-induced paw edema model.

5.2b.5 Effect of Aloe vera fractions on carrageenan -induced paw edema in rats

Inbred male rats (Rattus norvegicus) of Wistar strain reared in the Defence Food Research Laboratory animal facility, Mysore in the 150-200 g were allocated randomly to 7 groups of 6 animals each, group 1 (control group), group 2 (200 mg/kg body weight Aloe vera petroleum ether fraction group), group 3 (200 mg/kg body weight Aloe vera chloroform fraction group), group 4 (200 mg/kg body weight Aloe vera ethylacetate fraction group), group 5 (200 mg/kg body weight Aloe vera n-butanol fraction group), group 6 (200 mg/kg body weight Aloe vera water fraction group). All the rats were fed on standard rat feed and given free access to water. Twelve hours before the start of the experiment, rats were deprived of food, but given free access to water. Acute inflammation was produced by subplantar administration of 0.1 ml of 1%w/v carrageenan in the right paw of the rat. Fractions 200mg/kg body weight was administered to 18h fasted rats, intraperitoneally. After 60 min the inflammation is created by injecting carrageenan to paw of rats under anesthetic condition. The thickness (mm) of the paw was measured immediately and at 1, 2, 3, and 4hr intervals after the injection of the carrageenan (Dai et al., 2002; Winter et al., 1962). The paw volume was measured using digital Plethysmometer (UGO BASIL, Italy). Change in the paw volume was measured and anti-inflammatory activity was calculated by using the formula:
Percentage inhibition of oedema = 1 - \( \left( \frac{V_c - V_t}{V_c} \right) \times 100 \)

Where, \( V_c \) is the inflammatory increase in paw volume in control group of animals, \( V_t \) is the inflammatory increase in paw volume in Aloe vera fractions- treated animals.

### 5.2b.5 COLON ULCER

#### 5.2b.5a Treatment of experimental rats

This is earlier provided in the Chapter No. 3

#### 5.2b.5b Chemical, biochemical and enzymatic assays

This is earlier provided in the Chapter No. 4

#### 5.2b.5c Experimental protocol

Healthy male Wistar strain Albino rats inbred at Defence Food Research Laboratory animal house weighing approximately 110-150 g were allocated randomly to 8 groups of 6 animals each, Group 1 (control group), group 2 (6% acetic acid group), group 3 (50 mg/kg b.wt Aloe vera water fraction group), group 4 (50 mg/kg b.wt Aloe vera water fraction group + 6% acetic acid group), group 5 (100 mg/kg b.wt Aloe vera water fraction group), group 6 (100 mg/kg b.wt Aloe vera water fraction group + 6% acetic acid group), group 7 (200 mg/kg b.wt Aloe vera water fraction group) and group 8 (200 mg/kg b.wt Aloe vera water fraction group + 6% acetic acid group) received orally fed Aloe vera water fraction for 21 days. Groups 2, 4, 6 and 8 were administered intrarectally with 6% acetic acid and groups 1, 3, 5 and 7 were administered intrarectally with saline 24 hrs prior to sacrifice. All the animals were maintained on sterile, standard control diet and water, ad libitum. Food intake and weight gain were monitored weekly. On 21st day, all the rats were sacrificed under mild anesthesia and organs were quickly excised and stored in -20°C freezer until analyses. The experiments were designed and conducted after clearance from the Institutional Animal Ethical Committee, following the ethical guidelines for rats.
5.2b.5d Chemical analysis

The chemical analysis is as given in the Chapter No. 4

5.2b.5e Histopathological studies

The histopathological studies is as given in the Chapter No. 4

5.2b.6 GASTRIC ULCER

5.2b.6a Treatment of experimental rats

The treatment of animals is as given in the Chapter No. 3

5.2b.6b Chemical, biochemical and enzymatic assays

The chemical, biochemical and enzymatic assays is as given in the Chapter No. 4

5.2b.6c Experimental protocol

Healthy male Wistar strain Albino rats inbred at Defence Food Research Laboratory animal house weighing approximately 150-200 g were allocated randomly to 6 groups of 6 animals each, Group 1 (control), group 2 (ethanol), group 3 (20mg/kg b.wt omeprazole), group 4 (20mg/kg b.wt omeprazole + ethanol group), group 5 (200mg/kg b.wt Aloe vera water fraction group), group 6 (200mg/kg b.wt Aloe vera water fraction + ethanol group). Group 1 and 2 received orally distilled water, 3 and 4 received 20mg/kg b.wt omeprazole orally and 5 and 6 received 200mg/kg b.wt Aloe vera orally for 15 days. At the end of the experimental period, all the groups of rats were subjected to pylorus ligation.

Pylorus ligation in rats

The pylorus was ligated by means of the technique of Shay et al. (1945). Feed was withheld 12 hrs prior to the operative procedure. Group 1, group 4 and group 6 were orally fed with 1ml absolute alcohol. After few minutes, the rats were anaesthetized with ether and the abdomen was opened through a mid line incision. The pylorus was secured and ligated with silk sutures; proper care was taken not to
ligate the blood vessels. The abdominal walls were closed and the animals were allowed to recover from anesthesia. After pyloric ligation drinking water was withheld and the gastric juice was allowed to collect for a period of 8 hrs. The rats were then killed by an overdose of ether and the stomach was removed after clamping the oesophages.

5.2b.6d Chemical analysis

The stomachs were removed and gastric juice was collected. Later, the stomachs was opened along the great curvature and washed with tap water to remove gastric contents, and examined under a dissecting microscope with square-grid eyepiece to assess the formation of ulcers. Each stomach was opened along the greater curvature washed with saline unaware of experimental protocols and examined macroscopically for gastric erosions under a dissecting microscope (20 X). Lesions were also assessed by two observers unaware of experimental protocols. The length and width (mm) of ulcer on the gastric mucosa were measured by plane glass square (10×10 mm). The Ulcer Area (UA) was calculated. The % of protection (P%) availed to the animals through various treatments was calculated using the formula:

\[ P\% = \frac{UA_{ulcer\ control} - UA_{treatment}}{UA_{ulcer\ control}} \times 100 \]

The gastric mucosa was washed with 3 ml of lukewarm distilled water and collected in graduated centrifuge tubes. The gastric juice and washings were homogenized and centrifuged at (650 x g) for 5 min. The biochemical parameters assayed in gastric juice include carbohydrate content viz. fucose, hexosamine, total hexoses and total carbohydrates were evaluated. Secretions and enzymes viz. gastric volume, pH, free and total acidity, total proteins and pepsin activity were evaluated.

5.2b.6d.1 Gastric volume

This was measured after centrifuging the gastric fluid, allowed to stand, decant, and poured into the measuring cylinder of graduation 0.01 ml.
5.2b.6d.2 pH

The pH of the gastric juice was measured using the pH meter (Cyberscan, India)

5.2b.6d.3 Free acidity and total acidity

1 ml of gastric juice was pipetted into a 100 ml conical flask. Added 2 or 3 drops of Topfer’s reagent and titrated with 0.01N sodium hydroxide (NaOH) (which was previously standardized with 0.01 N of oxalic acid) until all traces of the red colour disappears and the colour of the solution was yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted. This volume corresponds to total acidity (Hawk et al., 1947). Acidity was calculated by using the formula,

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Actual Normality of NaOH} \times 100}{0.1} \text{ meq / 1/100 g}
\]

5.2b.6d.4 Total proteins

Proteins produce a violet color complex with copper ions in an alkaline solution. The absorbance of the color complex is directly proportional to the protein concentration in sample material. Lowry reagent was prepared by mixing sodium carbonate 100 ml, sodium potassium tartrate 1 ml and copper sulphate 1 ml. To the tissue homogenate 1 ml, 5 ml of Lowry reagent was added, mixed and incubated for 10 minutes at 25° C. Then FC reagent 0.5 ml was added and incubated for 30 min at 25° C. The absorbance of the sample was measured at 750 nm spectrophotometrically (Lowry et al., 1951). The amount of total protein in the tissue was expressed in mg protein/ml.
5.2b.6d.5 Total carbohydrates

The dissolved mucosubstances in gastric juice were estimated in the alcoholic precipitate obtained by adding 1 ml of gastric juice to 9 ml of 90% alcohol and the mixture was kept for 10 min and the supernatant was discarded. The precipitate separated was dissolved in 0.5 ml of 0.1 N sodium hydroxide. To this 1.8 ml of 6 N HCl was added. This mixture was hydrolyzed in the boiling water bath for 2 hrs (Goel et al., 1985). The hydrolysate was neutralized by 5 N sodium hydroxide using phenolphthalein as indicator and the volume was made up to 4.5 ml with distilled water and used for the estimation of total hexoses, hexosamine and fucose as described below.

5.2b.6d.5 Fucose

In this method, three test tubes were taken. In one tube 0.4 ml of distilled water was taken to serve as control and in each of the other two tubes 0.4 ml of hydrolysates were taken. To all three tubes 1.8 ml of sulphuric acid: water (6:1) was added by keeping the tubes in ice cold water bath to prevent breakage due to strong exothermic reaction. This mixture was then heated in boiling water bath for exactly 3 min. The tubes were then taken out and cooled. To the blank and to one of the hydrolysate containing tubes (unknown) 0.1 ml of cysteine reagent was added while cysteine reagent was not added to the last test-tube containing the hydrolysate (unknown blank). It was then allowed for 90 min to complete the reaction. The reading was taken in Hitachi 15-20 spectrophotometer at 396 and 430 nm setting the zero with the distilled water. The optical density for the fucose in the hydrolysate was calculated from the differences in the reading obtained at 296 and 430 nm and subtracting the values without cysteine. This was read against standard curve prepared with D (+) – fucose. The fucose content was expressed in terms of µg/ml of gastric juice (Dische and Shettles, 1948).

True optical density = $\frac{(OD_{396} - OD_{430})_{unknown} - (OD_{396} - OD_{430})_{unknown \; blank}}{(OD_{396} - OD_{430})_{water \; blank}}$
5.2b.6d.6 Hexosamine

0.5 ml of the hydrolysate faction was taken. To this 0.5 ml of acetyl-acetone reagent was added. The mixture was heated in boiling water bath for 20 min. Cooled under running tap water. 1.5 ml of 90% alcohol was then added followed by an addition of 0.5 ml of Ehrlich’s reagent. The reaction was allowed for 30 min. The colour intensity was measured in Hitachi 15-20 spectrophotometer at 530 nm against the blank prepared by using distilled water instead of hydrolysate. Hexosamine content of the sample was determined from the standard curve prepared by using D (+) glucosamine hydrochloride and concentration has been expressed in µg/ml of gastric juice (Dische and Barenfreund, 1950).

5.2b.6d.7 Total hexoses

To 0.4 ml of hydrolysate, 3.4 ml of orcinol reagent was added. The mixture was then heated in the boiling water bath for 15 min. This was then cooled under running tap water and intensity of the colour was read in Hitachi 15-20 spectrophotometer at 540 nm against the blank by using distilled water instead of hydrolysate. Total hexoses content was determined from the standard curve of D (+) – galactose has been expressed in µg/ml of gastric juice (Winzler, 1958).

5.2b.6e Histopathological studies

A portion of the ulcer region in the stomach tissue was dissected out and fixed in 10% buffered neutral formalin solution for histological observations. After fixation, tissue were embedded in paraffin, solid sections were cut at 5 µm and stained with hematoxylin and eosin (Gordon and Bradburg, 1990). The sections were examined with the help of a qualified pathologist under light microscope and photomicrographs were taken.

5.2b.7 ORAL ULCER

5.2b.7a Treatment of experimental rats

The treatment of animals is as given in the Chapter No. 3
5.2b.7b Chemical, biochemical and enzymatic assays

The chemical, biochemical and enzymatic assays is as given in the Chapter No. 4

5.2b.7c Experimental protocol

Healthy male Wistar strain Albino rats inbred at Defence Food Research Laboratory animal house weighing approximately 210-250 g were allocated randomly to 4 groups of 6 animals each, Group 1 (negative control oral ulcer untreated group), group 2 (positive control oral ulcer treated group), group 3 (200 mg/kg b.wt Aloe vera water fraction group) and group 4 (200 mg/kg b.wt Aloe vera water fraction group + oral ulcer treated group). Groups 2 and 4 rats were anesthetized with a mixture of 13 mg/kg of xylazine (2%) and 33 mg/kg of ketamine via intramuscular injection. After anesthesia, the tongue was pulled and a wound was inflicted in the central region, 3 mm from the tip, using a punch of 5 mm in diameter. The lesion had 1 mm in depth and was limited to the mucosa, without muscular involvement. Groups 3 and 4 rats were orally fed with Aloe vera water fraction and groups 1 and 2 were fed with physiological saline. The treatment lasts for 15 days. Twenty-four hours later after the last drug administration, rats were anesthetized with ether; oral mucosa was excised from the animal and stored at 20°C. Oral mucosa was centrifuged and supernatant was collected for biochemical analysis.

5.2b.7d Chemical analysis

The chemical analysis is as given in the Chapter No. 4

5.3 RESULTS AND DISCUSSION

5.3a Antioxidant activity of Aloe vera fractions

DPPH, a paramagnetic compound with an odd electron, shows strong absorption band at 517 nm. The absorbance decreases as a result of color change from purple to yellow due to the scavenging of free radical by antioxidants through donation of hydrogen to form the stable DPPH–H molecule (Arouma et al., 1997). The fractions are able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. Table 10 shows the scavenging effect of the Aloe vera fractions. From 25 fractions, petroleum ether (100), chloroform (100), ethylacetate (100), n-butanol (100), methanol (100), methanol: water (10:90), water (100) shows
highest scavenging effect. The experimental data of the Aloe vera fractions reveal that all the fractions possess the effect of scavenging free radical properties. The water fraction showed better radical scavenging activity than the other fractions.

As petroleum ether, chloroform, ethyl acetate, n-butanol, methanol and water fractions exhibited the highest scavenging activity in comparison with the other fractions; these five fractions were further studied. Aloe vera gel is rich in polysaccharides; glucomannans is one of the main polysaccharide present in Aloe vera gel.

5.3b Glucomannans in Aloe vera fractions

Glucomannans are polysaccharides that contain chains of randomly arranged $\beta$-(1→4)-D-mannose and $\beta$-(1→4)-D-glucose residues in a ratio of 3:1. Therefore, the fractions were used to study the amount of glucomannans present using Aloe vera glucomannans as standard. Fig 40 shows the amount of glucomannans present in each fraction. In this assay, water fraction showed highest when compare to other four fractions. Since, glucomannans is a water soluble polysaccharide might be the reason to be present more in water fraction. As Aloe vera polysaccharides have anti-inflammatory property, it was necessary to study the cyclooxygenase inhibitory activity of the fractions. Therefore, both in vitro and in vivo study have been studied in the Aloe vera fractions.

5.3c Effect of Aloe vera fractions on cyclooxygenase -2 inhibitory activity

Inflammation is the first response of the immune system to infection or irritation. It is caused by cytokines and eicosanoid such as prostaglandin E2. Cyclooxygenase or prostaglandin endoperoxide synthetase (COX) is the enzyme that catalyses the conversion of arachidonate to prostaglandins. COX enzyme performs its biological role in 2 isomers namely COX-1 and COX-2. In in vitro experiment, all the five fractions could provide a significant inhibition of COX-2 enzyme but water fraction showed the highest inhibition activity (61.15%) (Fig 41). COX-2 is induced at the site of inflammation and contributes to the inflammation process (Pairet and Enelhardt, 1996).
Table 10: Percentage yield of fractions of *Aloe vera* in different solvents

<table>
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<tr>
<th>Serial no.</th>
<th>Fractions analysed</th>
<th>Scavenging activity (%)</th>
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<tr>
<td>1.</td>
<td>petroleum ether (100)</td>
<td>52.78</td>
</tr>
<tr>
<td>2.</td>
<td>petroleum ether : choloroform (90 : 10)</td>
<td>40.56</td>
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<tr>
<td>3.</td>
<td>petroleum ether : choloroform (50 : 50)</td>
<td>40.27</td>
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<td>4.</td>
<td>petroleum ether : choloroform (10 : 90)</td>
<td>37.28</td>
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<tr>
<td>5.</td>
<td>choloroform (100)</td>
<td>50.00</td>
</tr>
<tr>
<td>6.</td>
<td>choloroform : ethylacetate (90 : 10)</td>
<td>11.79</td>
</tr>
<tr>
<td>7.</td>
<td>choloroform : ethylacetate (50 : 50)</td>
<td>40.56</td>
</tr>
<tr>
<td>8.</td>
<td>choloroform : ethylacetate (10 : 90)</td>
<td>38.52</td>
</tr>
<tr>
<td>9.</td>
<td>ethylacetate (100)</td>
<td>65.44</td>
</tr>
<tr>
<td>10.</td>
<td>ethylacetate : acetone (90 : 10)</td>
<td>11.79</td>
</tr>
<tr>
<td>11.</td>
<td>ethylacetate : acetone (50 : 50)</td>
<td>3.30</td>
</tr>
<tr>
<td>12.</td>
<td>ethylacetate : acetone (10 : 90)</td>
<td>8.57</td>
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<tr>
<td>13.</td>
<td>acetone (100)</td>
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<td>14.</td>
<td>acetone : n-butanol (90 : 10)</td>
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<td>15.</td>
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<td>37.27</td>
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<td>16.</td>
<td>acetone : n-butanol (10 : 90)</td>
<td>38.57</td>
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<td>17.</td>
<td>n-butanol (100)</td>
<td>54.32</td>
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<td>18.</td>
<td>n-butanol: methanol (90 : 10)</td>
<td>38.68</td>
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<td>19.</td>
<td>n-butanol: methanol (50 : 50)</td>
<td>38.43</td>
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<tr>
<td>20.</td>
<td>n-butanol: methanol (10 : 90)</td>
<td>39.17</td>
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<td>21.</td>
<td>methanol (100)</td>
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<td>methanol: water (50 : 50)</td>
<td>38.73</td>
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<td>24.</td>
<td>methanol: water (10 : 90)</td>
<td>58.17</td>
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<tr>
<td>25.</td>
<td>water (100)</td>
<td>89.97</td>
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Fig 40. Glucomannans in *Aloe vera* fractions

Values expressed as Mean ± SD of 3 individual samples (n=3)
It has been suggested that the selective inhibition of COX-2 isoform could be a relevant target for an anti-inflammatory drug because COX-1 maintains normal gastric mucosa and influences kidney functions (Vane and Botting, 1998). With the significant inhibition of COX-2, a desirable anti-inflammatory effect was clearly evident with *Aloe vera* fractions.

### 5.3d Effect of *Aloe vera* fractions on carrageenan–induced paw edema in rats

Further, experiments were conducted to assess the anti-inflammatory potential of *Aloe vera* fractions in acute model of inflammation for which carrageenan-induced edema was studied. Carrageenan induced inflammation is useful in detecting orally active anti-inflammatory agents (DiRosa et al., 1971). Oedema formation due to carrageenan in the rat paw is a biphasic event (Vinegar et al., 1969). The initial phase is attributed to the release of bradykinin, histamine and serotonin liberation by local cells, after a couple of hours there is liberation of prostaglandins (Crunkhon and Meacock, 1971). Table 11 gives the effect of percentage inhibition of *Aloe vera* fractions on paw oedema induced by carrageenan, where water fraction showed the highest inhibition (60.9%). The inhibition of prostaglandin formation through cyclooxygenase pathway is an established mechanism in carrageenan induced oedema. To reconfirm the involvement of cyclooxygenase inhibition, both *in vitro* and *in vivo* experiments were conducted.
Fig 41. Effect of *Aloe vera* fractions on cyclooxygenase inhibitor screening assay

Values expressed as Mean ± SD of 3 individual samples (n=3)

Table 11. Effect of administration of *Aloe vera* fractions on paw edema -induced by carrageenan (n=6)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>edema (ml)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-</td>
<td>0.72±0.020³</td>
<td>00.0</td>
</tr>
<tr>
<td>petroleum ether fraction</td>
<td>200</td>
<td>0.48±0.030³</td>
<td>33.3</td>
</tr>
<tr>
<td>chloroform fraction</td>
<td>200</td>
<td>0.45±0.030³</td>
<td>30.4</td>
</tr>
<tr>
<td>ethyl acetate fraction</td>
<td>200</td>
<td>0.37±0.030³</td>
<td>24.7</td>
</tr>
<tr>
<td>n-butanol fraction</td>
<td>200</td>
<td>0.39±0.030³</td>
<td>27.8</td>
</tr>
<tr>
<td>water fraction</td>
<td>200</td>
<td>0.22±0.001³</td>
<td>60.9</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD (n=6) (p<0.05)
5.3e Colon ulcer

Figs 42 and 43 presents the food intake and weight gain pattern of rats fed with Aloe vera fraction respectively. The results show that the feeding of the Aloe vera water fraction or the administration of acetic acid did not affect the daily food intake and weight gain of rats. Table 12 presents the organ weights viz. liver, kidney, heart, brain and colon. The results show that the feeding of Aloe vera fraction or the administration of acetic acid did not affect weight of the organs.

Figs 44, 45, 46, 47, 48 and 49 gives the colonic enzyme and antioxidant /detoxifying enzyme viz. MPO, catalase, SOD, GST, GSH-Px and G-6-PD respectively. MPO activity in colon did not show any change by the feeding of Aloe vera water fraction per se. There was also significant decrease in colonic GSH-Px with associated decrease in G-6-PD and GST activities. The combined administration of acetic acid and Aloe vera fraction (200 mg/kg b.wt.) showed the reversal of acetic acid-induced myeloperoxidase, GSH-Px, G-6-PD and GST activities. A decline in colonic catalase and SOD activities was also noted in the group of rats administered with acetic acid and Aloe vera fraction (200 mg/kg b.wt).

Figs 50, 51, 52, 53 and 54 gives the effect of feeding of Aloe vera fraction on acetic acid-induced changes in hepatic GSH-Px, GST, SOD, catalase and G-6-PD respectively. Administration of acetic acid reduced the hepatic catalase, GSH-Px, SOD and G-6-PD activities and there was a rise in the hepatic GST activity significantly.
Fig 42. Effect of *Aloe vera* water fraction on acetic acid-induced changes on food intake of rats (n=6)

Values are expressed as Mean ± SD

Fig 43. Effect of *Aloe vera* water fraction on acetic acid-induced changes on weight gain of rats (n=6)

Values are expressed as Mean ± SD
Table 12. Effect of *Aloe vera* water fraction on acetic acid -induced changes on organ weights of rats (n=6)

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>Colon (g/100g body wt)</th>
<th>Liver (g/100g body wt.)</th>
<th>Kidney (g/100g body wt.)</th>
<th>Heart (g/100g body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.35±0.011</td>
<td>3.89±0.12</td>
<td>1.134±0.123</td>
<td>0.336±0.032</td>
</tr>
<tr>
<td>6% Acetic acid</td>
<td>0.40±0.027</td>
<td>3.95±0.10</td>
<td>1.890±0.142</td>
<td>0.306±0.0120</td>
</tr>
<tr>
<td>50mg <em>Aloe vera</em> fraction</td>
<td>0.34±0.031</td>
<td>3.22±0.10</td>
<td>1.223±0.113</td>
<td>0.342±0.012</td>
</tr>
<tr>
<td>50mg <em>Aloe vera</em> fraction + Acetic acid</td>
<td>0.33±0.045</td>
<td>3.74±0.17</td>
<td>1.235±0.102</td>
<td>0.350±0.043</td>
</tr>
<tr>
<td>100mg <em>Aloe vera</em> fraction</td>
<td>0.32±0.017</td>
<td>3.28±0.52</td>
<td>1.229±0.116</td>
<td>0.340±0.012</td>
</tr>
<tr>
<td>100mg <em>Aloe vera</em> fraction + Acetic acid</td>
<td>0.35±0.012</td>
<td>3.87±0.12</td>
<td>1.242±0.132</td>
<td>0.345±0.023</td>
</tr>
<tr>
<td>200mg <em>Aloe vera</em> fraction</td>
<td>0.32±0.031</td>
<td>3.27±0.17</td>
<td>1.221±0.117</td>
<td>0.344±0.010</td>
</tr>
<tr>
<td>200mg <em>Aloe vera</em> fraction + Acetic acid</td>
<td>0.31±0.029</td>
<td>3.78±0.25</td>
<td>1.238±0.100</td>
<td>0.348±0.021</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD

Fig 44. Effect of *Aloe vera* water fraction on acetic acid -induced changes in myeloperoxidase activity in rats

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)
Fig 45. Effect of *Aloe vera* water fraction on acetic acid-induced changes in colonic catalase activity in rats

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)

Fig 46. Effect of *Aloe vera* water fraction on acetic acid-induced changes in colonic superoxide dismutase activity in rats

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)
Fig 47. Effect of *Aloe vera* water fraction on acetic acid-induced changes in colonic glutathione S-transferase activity in rats

Values Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)

Fig 48. Effect of *Aloe vera* water fraction on acetic acid-induced changes in colonic glutathione peroxidase activity in rats

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)
Fig 49. Effect of *Aloe vera* water fraction on acetic acid-induced changes in colonic glucose-6-phosphate dehydrogenase activity in rats

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)

Fig 50. Effect of *Aloe vera* water fraction on acetic acid-induced changes in hepatic glutathione peroxidase activity in rats

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)
Conventional corticosteroids have been used as a major component of acute inflammatory bowel disease management; however they have many disadvantages (Robinson, 1998). Hence herbal therapies based on the plant formulation are widespread and increasing as complementary and alternative therapies for inflammatory bowel disease (IBD) (Langmead and Rampton, 2006). Administration of acetic acid resulted in colonic inflammation in the experimental rats characterized by necrosis of the mucosa extending along the colon. In the present study, increased myeloperoxidase enzyme activity, the marker enzyme of the neutrophil confirms the successful induction of colitis in colon. Myeloperoxidase is an important enzyme of neutrophils. It is the key enzyme for the formation of hypochlorous acid from \( \text{H}_2\text{O}_2 \) in the presence of chloride ions. Hypochlorous acid is a potent oxidant known to have several cytotoxic effects on bacterial cells. The integrity of bacterial cell membrane may be violated by the oxidation of membrane proteins (Sato et al., 1999). Additionally, activated neutrophils and monocytes can also generate cytotoxic chloramines, tyroxyl radicals, and \(-\text{OH}\) via a myeloperoxidase-dependent pathway (Hampton et al., 1998; Miller and Britigan, 1997). The increased concentration of lipid peroxidation products (MDA) as a result of the induction of colitis may be due to the generation of increased ROS with impaired antioxidant defence mechanism.

Catalase is a haemo enzyme, which removes \( \text{H}_2\text{O}_2 \) and GSH-Px, a selenium-containing enzyme scavenges \( \text{H}_2\text{O}_2 \) and other peroxides (Halliwell and Gutteridge, 1989). During oxidative stress, catalase activity decreases, hydrogen peroxide accumulates and thereby more peroxidation of lipids is favoured (Rodriguez-cabezas et al., 2002). GSH exhibits its antioxidant effect by reducing with superoxide radical and hydroxyl radical following the formation of oxidized glutathione (Beckman and Ames, 1997) and reduces peroxides in the non-enzymatic reaction (Korkina et al., 2003). The observed decrease in colonic GSH may be due to increased conjugation reaction with acetic acid. Recently we have reported the potent antioxidant activity of *Aloe vera* gel extracts (Saritha et al., 2010).

The present study demonstrated that *Aloe vera* water fraction enhances the catalase activity in colon and liver samples of the rats. Increased GSH-Px in colon and liver observed in the *Aloe vera* water fractions group may help reducing the onset of peroxidative stress. Superoxide dismutase is responsible for removal of superoxide radical (Nishikimi et al., 1972), thus it may contribute to the modulation of redox state of liver cells.
Fig 51. Effect of *Aloe vera* water fraction on acetic acid-induced changes in hepatic glutathione S-transferase activity in rats

Values are Mean ± SD for 6 rats.
Values bearing different superscripts in the same column are significantly different (p<0.01)

Fig 52. Effect of *Aloe vera* water fraction on acetic acid-induced changes in hepatic superoxide dismutase activity in rats

Values are Mean ± SD for 6 rats.
Values bearing different superscripts in the same column are significantly different (p<0.01)
Fig 53. Effect of *Aloe vera* water fraction on acetic acid-induced changes in hepatic catalase activity in rats

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)

Fig 54. Effect of *Aloe vera* water fraction on acetic acid-induced changes in hepatic glucose-6-phosphate dehydrogenase activity in rats

Values are Mean ±SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)
Table 13. Effect of *Aloe vera* fraction on acetic acid -induced changes in colonic antioxidant and lipid peroxidation

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>GSH (µmoles/g)</th>
<th>MDA (nmoles/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.8±1.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.5±12.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6% Acetic acid</td>
<td>27.9±1.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>205.1±10.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>50mg <em>Aloe vera</em> fraction</td>
<td>42.7±6.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135.6±13.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50mg <em>Aloe vera</em> fraction+ Acetic acid</td>
<td>25.5±3.04&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>168.0±14.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 mg <em>Aloe vera</em> fraction</td>
<td>44.8±3.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>137.3±12.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100mg <em>Aloe vera</em> fraction+ Acetic acid</td>
<td>27.4±1.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>159.2±16.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>200 mg <em>Aloe vera</em> fraction</td>
<td>47.9±7.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>139.2±14.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200mg <em>Aloe vera</em> + Acetic acid</td>
<td>32.5±5.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>150.6±15.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)

Table 14. Effect of *Aloe vera* fraction on acetic acid -induced changes in hepatic antioxidant and lipid peroxidation

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>GSH (µmoles/g)</th>
<th>MDA (nmoles/g)</th>
<th>CDx10&lt;sup&gt;-1&lt;/sup&gt; nmoles/g</th>
<th>HP moles/gx 10&lt;sup&gt;-5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.4±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.201±0.010&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6% Acetic acid</td>
<td>05.6±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.17±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.311±0.030&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.01±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>50mg <em>Aloe vera</em> fraction</td>
<td>11.8±1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.15±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.190±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.01±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50mg <em>Aloe vera</em> fraction+ Acetic acid</td>
<td>09.9±0.68&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>4.52±0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.222±0.010&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.01±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100mg <em>Aloe vera</em> fraction</td>
<td>11.9±1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.18±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.189±0.011&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.04±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100mg <em>Aloe vera</em> fraction+ Acetic acid</td>
<td>09.9±0.53&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>4.14±0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.220±0.050&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.02±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200mg <em>Aloe vera</em> fraction</td>
<td>11.9±1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.19±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.210±0.011&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.03±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200mg <em>Aloe vera</em> + Acetic acid</td>
<td>09.9±0.50&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>4.03±0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.200±0.012&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.01)
The enhanced G-6-PD may play a significant role in elevating the GSH level in colon. The increase in G-6-PD activity observed due to the consumption of *Aloe vera* water fraction leads to elevate the hexose monophosphate shunt (HMP shunt) pathway of carbohydrate metabolism and there by increases the level of NADPH, which in turn helps to keep a raised level of reduced glutathione from the oxidized state. Enhancement of GST has been shown to increase the ability for detoxification of some carcinogens (Stavric, 1994).

Table 13 gives the effect of *Aloe vera* water fraction on acetic acid-induced changes on colonic antioxidant. The administration of acetic acid resulted in significant reduction in colonic GSH with an associated increase in MDA content. The feeding of *Aloe vera* water fraction per se did not result in any change in colonic GSH and MDA content. The increased concentration of lipid peroxidation products (MDA) as a result of the induction of colitis may be due to the generation of increased ROS with impaired antioxidant defence mechanism. Thiols are thought to play a vital role in protecting cells against lipid oxidation (Pryor, 1973). The *Aloe vera* water fraction has been found to be effective in increasing the GSH content rendering the protection against acetic acid- induced hepatic and colonic lipid peroxidation.

Table 14 presents effects of *Aloe vera* water fraction feeding on acetic acid-induced hepatic antioxidant viz. glutathione and hepatic lipid peroxides contents viz. MDA, conjugated dienes, hydroperoxides. The *Aloe vera* fractions at 50, 100, 200 mg/kg b.wt increased the hepatic GSH contents as compared to control group of rats. The co-administration of acetic acid with *Aloe vera* fraction at all the doses significantly decreased. It is reported that the functions of GSH in reductive processes are essential for the synthesis and also degradation of proteins, regulation of enzymes and protection of the cell against reactive oxygen species and free radicals produced evening normal metabolis (Meister and Anderson, 1983; Sen, 1997). The significant livers hepatic GSH weight is helpful in providing a cleopholic environment in the cell so that the attack of the oxidants from the pesticide is resisted. GSH represents an important defence mechanism in protecting cells against oxygen free radicals (Linder, 1985). The synthesis of this endogenous tripeptide may also be influenced by a change in availability of cysteine derived for dietary cysteine, protein breakdown or methionine via the transformation pathway (Tateishi et al., 1974; Beatly and Reed,
The diet was supplied with adequate amount of methionine. The rate of efflux of hepatic GSH into plasma may influence the tumor rate of GSH in the liver (Lauterburg et al., 1984).

MDA is known to be a modulator of signal transduction pathway that disturbs cellular activity (Grunes et al., 1997). The administration of acetic acid resulted in an increase in hepatic MDA content. The intake of Aloe vera fractions at 50, 100, 200mg/kg b.wt with HCH treatment has also increased the MDA content. The prefeeding of Aloe vera resulted in a decreased MDA content as compared to control group of rats. Conjugated dienes contents are significantly high in acetic acid-induced rats in comparison to control group rats. The co-administration of acetic acid with Aloe vera fraction at all the levels showed significant decrease in conjugated dienes contents as compared to acetic acid group rats. The hydroperoxides contents are significantly increased in acetic acid-induced group of rats. The prefeeding of Aloe vera fractions in all the three doses with acetic acid treatment also increased the hydroperoxide contents but in comparison to acetic acid-induced rats, it showed decreased hydroperoxide contents. The lipid peroxidation, as observed in this study is a molecular mechanism of cell injury leading to generation of peroxides and lipid hydroperoxides which can decompose to yield a range of cytotoxic products, most of which are aldehyde, as exemplified by MDA, 4-hydroxynonenal, etc. (Esterbauer et al., 1991). The situation of lipid peroxidation as a consequence of tissue injury can sometimes make significant contribution to worsening of injury. This study shows that the Aloe vera water fraction is highly efficient in the reduction of in situ generated toxic lipid peroxides.

**Histopathology studies**

Severity of the inflammation in colon, liver, kidney, small intestine and heart was assessed and graded semi quantitatively (Al-Awadi et al., 2001) as follows: Grade 1- mild acute inflammation in wall, Grade 2- moderate acute inflammation, small ulcers and Grade 3- severe acute inflammation+ulceration/necrosis of wall. Results of histopathological examinations showed that control group animals did not show any symptoms for inflammation whereas, acetic acid treated group showed moderate acute inflammation, small ulcers and some showed severe acute
inflammation + ulceration/necrosis of wall. The water fraction of *Aloe vera* treated group showed reduced intensity of lesions in colon alone without any evidence of necrosis and regeneration or inflammatory reaction (200 mg/kg body wt.) (Fig 55). The interpretation of the histology results were done with the support of a Pathologist.

### 5.3f Gastric ulcer

Table 15 shows the pH, total gastric volume, free acidity, total acidity, ulcer area and protection in gastric juice of rats. A high degree of ulcer area was obtained in ethanol-induced group of rats when compared to control animals. A decline in ulcer area and significant protection was noted in the group of rats administered with ethanol and *Aloe vera* fraction. Figs 56, 57, 58, 59 and 60 shows the levels of glycoprotein contents and TC: P ratio in the gastric juice. From the results it is evident that the levels of hexose, hexosamine, sialic acid, fucose and TC: P ratio. Oral administration of *Aloe vera* fraction significantly decrease the levels of glycoprotein contents.

It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms (Szabo and Szienji, 1987). The excess gastric acid formation by prostaglandin includes both increases in mucosal resistance as well as decrease in aggressive factors, mainly acid and pepsin (Aly, 1987). Gastric mucus plays an important role in gastric defensive mechanisms by acting as a protective barrier, mainly because of its glycoprotein content. The water stored by these glycoprotein prevents hydrogen ions from reaching the cell surface (Motivla et al., 1996).
Fig 55. Micrographs showing the histopathological analysis of the colonic tissue in rats

a: control group, no acute inflammation. b: 6% acetic acid group, severe acute inflammation + ulceration/necrosis of wall. c: *Aloe vera* methanol extract (50mg/kg b.wt) group, no acute inflammation. d: *Aloe vera* methanol extract (50mg/kg b.wt) + acetic acid group. Moderate acute inflammation ± small ulcers. e: *Aloe vera* methanol extract (100mg/kg b.wt) group, no acute inflammation. f: *Aloe vera* methanol extract (100mg/kg b.wt) + acetic acid group, moderate acute inflammation ± small ulcers. g: *Aloe vera* methanol extract (200mg/kg b.wt) group, no acute inflammation. h: *Aloe vera* methanol extract (200mg/kg b.wt) + acetic acid group, mild acute inflammation in wall or serosa.
(e) *Aloe vera* water fraction group (100mg/kg b.wt)

(f) *Aloe vera* water fraction + acetic acid group (100mg/kg b.wt)

(g) *Aloe vera* water fraction group (200mg/kg b.wt)

(h) *Aloe vera* water fraction + acetic acid group (200mg/kg b.wt)
Consumption of alcohol produce severe hemorrhagic lesions in the gastric mucosa and hence ethanol induced ulcer model was included in the present study. The factors involved in the formation of ulcer using ethanol have been described (Lang et al., 1985). Hypoacidity was defined as pH level of gastric juice greater than 3.5 (Hsu et al., 2007). The color of gastric juice was carefully assessed, and bile stain of gastric juice was defined as yellowish or greenish discoloration of the gastric juices. The inhibition of acid secretion is the one of most important factors for the healing of gastric ulcers. In the pylorus ligative model, the \textit{Aloe vera} water fraction significantly reduced gastric acidity \([H^+]\) at a dose of 200 mg/kg body wt. as did omeprazole, when compared to control group. These results show that a possible antisecretory effect is involved in the antiulcerogenic activity of the \textit{Aloe vera} water fraction. The hyposecretory nature of \textit{Aloe vera} fraction in ulcer-induced rats may further help in decreasing the volume, pH and acidity of gastric juice towards near normal levels. Thus normalization of gastric juice acidity may indirectly help in healing of ulcer lesions in \textit{Aloe vera} treated ulcer rats.

In addition, the ulcer index decreased after treatment with the \textit{Aloe vera} water fraction without any change in the volume of gastric secretions as also found to omeprazole.

Mucin is a viscous glycoprotein with physiochemical properties producing relatively resistant acid barrier (Flemstrong and Garner, 1982). Glycoproteins are the important constituents of plasma membrane and specific intracellular organelles such as golgi complexes, lysosomes and secretory granules. Surface mucus cells and mucus neck cells of gastric mucosa secrete mucus by exocytosis (Zalewsky and Moody, 1979). The main components of gastric mucus are the acidic glycoprotein, sialic acid and neutral mucopolysaccharides viz. total hexoses, hexosamine and fucose. These glycoproteins are of importance for their specific properties such as gel formation and viscosity. Glycoproteins are obligatory components of mucus and their quantitative determination has been used as a measure of mucus formation (Lukie and Frostner, 1972). A decrease in the synthesis of sulphated mucus glycoprotein has been implicated in the etiology of peptic ulcer (Younan et al., 1982).
Table 15. Effect of *Aloe vera* fraction on ethanol-induced changes on pH, gastric volume, free acid and total acid, ulcer area and protection in rat

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>pH (unit)</th>
<th>Total gastric volume (ml)</th>
<th>Free acidity (mEqL⁻¹)</th>
<th>Total acidity (mEqL⁻¹)</th>
<th>Ulcer area (mm²)</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.28 ± 0.04ᵃ</td>
<td>2.63 ± 0.68ᵃ</td>
<td>1.36 ± 0.08ᵃ</td>
<td>3.63 ± 0.14ᵃ</td>
<td>000.00±0.00</td>
<td>NA</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.56 ± 0.24ᵇ</td>
<td>4.78 ± 0.57ᵇ</td>
<td>3.33 ± 0.10ᵇ</td>
<td>6.62 ± 0.23ᵇ</td>
<td>790.66±6.43</td>
<td>0.0</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>4.24 ± 0.50ᵃ</td>
<td>2.72 ± 0.26ᵃ</td>
<td>1.31 ± 0.10ᵃ</td>
<td>3.24 ± 0.28ᵃ</td>
<td>000.00±0.00</td>
<td>NA</td>
</tr>
<tr>
<td>Omeprazole + ethanol</td>
<td>4.02 ± 0.60ᶜ</td>
<td>2.74 ± 0.23ᶜ</td>
<td>2.39 ± 0.20ᶜ</td>
<td>4.98 ± 0.53ᶜ</td>
<td>162.33±10.40</td>
<td>75.60%</td>
</tr>
<tr>
<td><em>Aloe vera</em> fraction</td>
<td>4.34 ± 0.77ᵃ</td>
<td>2.90 ± 1.27ᵃ</td>
<td>1.64 ± 0.31ᵃ</td>
<td>3.77 ± 0.31ᵃ</td>
<td>000.00±0.00</td>
<td>NA</td>
</tr>
<tr>
<td><em>Aloe vera</em> fraction + ethanol</td>
<td>3.97 ± 0.54ᶜ</td>
<td>3.00 ± 1.08ᶜ</td>
<td>2.33 ± 0.14ᶜ</td>
<td>5.05 ± 0.36ᶜ</td>
<td>237.45±21.54</td>
<td>68.92%</td>
</tr>
</tbody>
</table>

Values are Mean ± SD for 6 rats.
Values bearing different superscripts in the same column are significantly different (p<0.05)
The two main features of the mucus layer are its thickness and turnover rate. These two processes are of great value in protecting the mucosal layers from the offensive factors (Allen, 1978). The mucosal layer is a dynamic entity in which the surface cells are continuously renewed (Bickel and Kauffmen, 1981). The observed decrease in the levels of sialic acid and hexosamine levels may be attributed to the decreased production or turnover mucus. The mucus possessing fewer amounts of sialic acid and hexosamine is prone for easy degradation (Kerss et al., 1982). Prefeeding of Aloe vera fraction antagonizes the aggressive factors, which plays a crucial role in the pathogenesis of gastric lesions and augment defensive factors to protect the gastric mucosa from ulceration. The increase in the levels of glycoproteins, particularly sialic acid and hexosamine in the Aloe vera water fraction treated groups indicate the increase in the production of mucus, thereby possibly protecting the gastric mucosa in ulcer models. The efficacy of Aloe vera fraction showed promising antiulcer activity less than that of omeprazole.

The observed decrease in the levels of glycoprotein moieties in gastric juice of ulcerated groups of rats may be attributed due to disintegration and degradation of glycoprotein moieties into their simpler components in the process of ulceration induced by ulcerogens. The histopathological observations made on the stomach tissue support this view. 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 (Suvitayavat et al., 2004). Thus the present investigation establishes the gastro-protective nature of Aloe vera water fraction and the protective effect may be mediated by defensive mucosal factors.
Fig 56. Effect of water fraction of *Aloe vera* against ethanol-induced changes in hexoses of rat gastric juice

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.05)

Fig 57. Effect of water fraction of *Aloe vera* against ethanol-induced changes in hexosamine of rat gastric juice

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.05)
Fig 58. Effect of water fraction of *Aloe vera* against ethanol-induced changes in sialic acid of rat gastric juice

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.05)

Fig 59. Effect of water fraction of *Aloe vera* against ethanol-induced changes in fucose of rat gastric juice

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.05)
Figure 60. Effect of water fraction of *Aloe vera* against ethanol-induced changes in total protein of rat gastric juice

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.05)

Fig 61. Effect of water fraction of *Aloe vera* against ethanol-induced changes in TC:P ratio of rat gastric juice

TC:P ratio: total carbohydrate : protein ratio

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.05)
Histopathological studies

The results on histopathological investigations revealed that the pretreatment with omeprazole and *Aloe vera* water fraction absolutely prevented the ethanol-induced congestion, haemorrhage, edema, necrosis, inflammatory and dysplastic changes erosions and ulceration in the gastric mucosa of rats. The results were interpreted with the support of Pathologist (Figs 62 and 63).

5.3g Oral ulcer

Wound healing constitutes a perfectly coordinated cascade of cellular and molecular events that interact in order to promote tissue repair. Various authors have investigated medications that might accelerate wound healing, reduce painful symptoms associated with oral ulcers and have an optimal cost-benefit ratio. Oral mucosal wound or mouth ulcers are open lesions within the mouth, caused by various disorders. The early lesions are vesicles which can affect any part of the oral mucosa. The vesicles are domeshaped and usually 2-3mm in diameter. Rupture of vesicles leaves circular, sharply defined, shallow ulcers with yellowish or grayish floors and red margins. The ulcers are painful and may interfere with eating. The cause of some oral mucosal ulcers may be related to a temporary weakness in immune system (for example, from cold or flu), hormonal changes, mechanical irritation, stress, low levels of vitamin B\textsubscript{12}, folate, iron and ferritin (Yeung-Yue, 2002; Greenberg and Pinto, 2003).

Table 16 illustrates the effect of water fraction of *Aloe vera* on the mucosa SOD, GSH-Px activity and MDA level in experimental groups of rats. Ulcer model rats produced a significant decrease SOD, GSH-Px activity and increase MDA level in the mucosa in ulcer model rats, when compared with normal rats. Administration of water fraction of *Aloe vera* significantly enhanced SOD, GSH-Px activity and reduced MDA level in the mucosa in *Aloe vera* fraction + ulcer model when compared to ulcer model rats. The cellular antioxidant defense system operates through enzymatic and nonenzymatic components. SOD is the necessary enzymes for an effective defense against ROS (Chen et al., 2008). SOD accelerates the formation of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) by using superoxide radicals. CAT is the enzyme that accelerates the degradation of unstable H\textsubscript{2}O\textsubscript{2} to H\textsubscript{2}O and O\textsubscript{2} (Dugan et al., 1995; Luo and Fang, 2008; Yu et al., 2009).
Fig 62. The effects of the water fraction of *Aloe vera* on ethanol-induced changes in rats (macrography). The mucosal layer injury of the gastric tissue obtained from the untreated rats with control (A), treated rats with ethanol (B), treated rats with a standard drug omeprazole + ethanol (C) and treated rats with water fraction of *Aloe vera* water fraction + ethanol (D). The mucosal injuries of C and D are dramatically reduced as compared to that of B.
Fig 63. Micrographs showing the histopathological analysis of the stomach tissue in rats.

a: control group, normal gastric mucosa with normal glands, submucosa and muscularis unremarkable. b: ethanol group, large area of mucosal ulceration with diffuse inflammatory infiltrate seen with areas of haemorrhage and many congested blood vessels seen. c: omeprazole group, normal gastric mucosa with normal glands. d: omeprazole group + ethanol group, normal gastric mucosa with normal glands, submucosa and muscularis unremarkable. e: Aloe vera water fraction (200mg/kg b.wt) group, normal gastric mucosa with normal glands, submucosa and muscularis unremarkable. f: Aloe vera water fraction (200mg/kg b.wt) + ethanol group, normal gastric mucosa with normal glands, submucosa and muscularis unremarkable.
Table 16. Effect of Aloe vera fraction on oral ulcer-induced changes in antioxidant/detoxifying enzymes/lipid peroxidation

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>MDA nmoles/g</th>
<th>SOD *</th>
<th>GSH-Px ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>08.14 ± 0.53^a</td>
<td>221.46 ± 15.11^a</td>
<td>202.23 ± 11.08^a</td>
</tr>
<tr>
<td>Ulcer model</td>
<td>14.94 ± 1.08^b</td>
<td>121.32 ± 13.77^b</td>
<td>113.16 ± 10.34^b</td>
</tr>
<tr>
<td>Aloe vera fraction</td>
<td>08.04 ± 0.23^a</td>
<td>220.23 ± 12.01^a</td>
<td>200.79 ± 06.15^a</td>
</tr>
<tr>
<td>Aloe vera fraction + ulcer model</td>
<td>07.41 ± 0.72^a,c</td>
<td>217.32 ± 15.36^a,c</td>
<td>177.13 ± 04.12^a,c</td>
</tr>
</tbody>
</table>

* units / min / mg protein; *** m moles NADP reduced / min / mg protein

Values are Mean ± SD for 6 rats.

Values bearing different superscripts in the same column are significantly different (p<0.05)