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Ameliorative Effect of *Achillia Mellifolium* Extract in Experimentally Induced Ulcers in Rats.

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Abstract

Problem: Peptic ulcer disease is the most prevalent gastrointestinal disorder and objective of the present study was to evaluate *A. Mellifolium* plant extract for its antioxidant and antiulcer activity on pylorus ligation induced ulcers in rat stomach.
Experimental Approach: The methanol extracts of*A. Mellifolium* plant was used to assess MPO, SOD, TBARS, GSH and NO levels in pylorus ligation ulcers in rat stomach.
Findings: Results of present studies revealed that, pretreatment with *A. Mellifolium* at a dose of (100 mg/kg/*p.o.* and 125mg/kg/*p.o*) produced a dose dependent decrease in ulcer index in pylorus ligation-induced ulcers. Pre-treatment with *A. Mellifolium* (100 mg/kg/*p.o.* and 125mg/kg/*p.o*) markedly prevented the oxidative stress by improving the integrity of stomach, increasing the concentration of tissue nitrite/nitrate, SOD, GSH and by decreasing the level of TBARS and MPO in pylorus ligation induced ulcers models. 100mg/kg of *A. Mellifolium* showed marked improvement in all parameters in comparison to 125mg/kg. However, treatment with *A. Mellifolium* (100 mg/kg/*p.*o and 125mg/kg/p.o) improved the level of gastric adhesion mucus content in pylorus ligation-induced ulcers.
Conclusion: The present findings suggest that, extracts of *A. Mellifolium* extract possessAmeliorative effect against pylorus ligation induced ulcers models. The plant extract of *A. Mellifolium* (methanol) is a potential source of natural antioxidants for the treatment and prevention of disease in which oxidative stress is to be increased.
Keywords: *A. Mellifolium,* TBARS, GSH, SOD, NO.

Introduction

Peptic ulcer disease is the most prevalent gastrointestinal disorder (Onasanwo *et al*., 2011). It is to be characterized as deep lesions that penetrate through the entire thickness of the gastrointestinal tract (g.i.t) including mucosa and muscularis mucosa that develop due to exposure high gastric juice secretions to stomach. The most prominent cause of peptic ulcer is infection with the bacterium called Helicobacter pylori(H. pylori) and the use of drugs like Non Steroidal Anti-Inflammatory Drugs (NSAIDs) (aspirin and ibuprofen) (Kumar *et al*., 2011). It has been reported that imbalance between gastric offensive factors (pepsin, lipid Peroxidation, nitric oxide) and defensive factors (mucin secretion, glycoprotein and glutathione) are too responsible for ulceration (Oh *et al*., 2008). Various factors that play a pivotal role in the pathogenesis of ulcerations like sedentary life style, alcohol intake, spicy food, NSAID and various bacterial infections like *H. pylori* (Gisbert *et al*., 2004). When patients taking NSAIDs were excluded (Gisbert *et al*., 2004). Aggressive acid secretion has been reported to play a progressive role in gastric ulceration (Dharmani *et al*., 2003). Parietal cells are basically involved in the secretion of acid and in the generation of the hydrogen ion (H^+) (Petronic *et al*., 2002). Impairment of gastric ulcer healing depends upon the augmented release of pro-inflammatory cytokines (Harsch *et al*., 2003). Literature review revealed that oxidative stress too plays a pivotal role in progression of ulcer that directly impaired cells functions (Tandon *et al*., 2004). Experimental and clinical studies suggested that the reactive oxygen species (ROS) and the reactive nitrogen species (RNS) have an important role in the aetio-pathogenesis of theinflammation and ulceration of the digestive tract (Adriana *et al*., 2008). ROS plays a vital role in apoptotic process, which involves the release of proteins that triggers the activation of caspases-3, caspases-4 and Cytochrome c. (Wang *et al*., 2003; Yaguchi *et al*., 2010). Matrix metallprotinase (MMPs) an enzyme plays a major role in the ulcer tissue remodelling (Mignatti *et al*., 1996; Tomita *et al*., 2009). NO generated from the endothelial nitric oxide synthase (e-NOS) plays an important role in ulcer healing by promoting angiogensis regulates gastric mucosal blood flow and stimulates gastric mucus secretion (Nishida *et al*., 1997; Li and Wallace, 2000; Pan *et al*., 2005). Further, Growth factors are too implicated in ulcer healing (Milani and Calabro, 2001). It has been shown that various experimental methods are available for the induction of ulcer which includes ethanol-induced ulcers, pylorus ligation-induced ulcers, stress-induced ulcers, acetic acid-induced ulcers and reserpine-induced gastric ulcers (Jain and Surana, 2009; Srinivas and Baboo, 2011; Khan *et al.,* 2011). Further, histamine (H), cysteamine and serotonin (5-HT) (Nassar *et al.,* 1987) and smocking (Ma *et al*., 1999) are too used as experimental models for induction of ulceration. Further smoking is also major cause associated with ulcer. In addition to this various poly-herbal formulations are available in the market but standardization of these polyherbal formulation in order to get a standard product repeatedly is not an easy task (Bafna and Balaraman, 2005; Shirwaikar *et al.,* 2006; Darbar *et al.,* 2010). Bioactivity of this plant, recent studies reported antimicrobial, antiphlogistic, hepatoprotective, antispasmodic and calcium antagonist activities of its polar extracts (Stojanović *et al.,* 2005; Yaeesh *et al.,* 2006), and a protective effect of its infusions against H_2O_2- induced oxidative damage in human erythrocytes and leucocytes (Konyalioglu & Karamenderes, 2005).
But still no clinical study has been conducted to explore its antiulcer activity of *A. Mellifolium*.

Thus the present study has been designed to explore ulcerprotective and antioxidant activity of *A. Mellifolium*in pyloric ligation-induced ulcers in rats.

Materials and Methods

The experimental protocol used in present study was approved by Institutional Animal Ethical Committee (IAEC). Age matched young wistar rats weighing 200-250 g were employed in the study. The animals were fed on standard chow diet and water *ad libitum*. They were acclimatized in the animal house of our institute and exposed to natural light and dark cycle.

Plant material

Mellifolium was obtained as gift sample from AMSAR Pvt. Ltd. DTNB was purchased from Sanjay biological Amritsar, India. NEDA and TCA were purchased from (SDFCL) S D Fine-Chem Ltd, Mumbai. TBA was purchased from Loba Chemie Pvt Ltd., Mumbai and Sulfanilamide was purchased from Titan Biotech Limited. All other reagents used in the present study were of analytical grade.

Preparation of extract

The leavesof the *A. Mellifolium* was washed with water, air- dried and grounded into fine powder the extract was prepared in ethanol by using soxhlet apparatus. The Methanol extract was concentrated by evaporating it on water bath at 50°c. The concentrated methanol extract were then used for study.

Phytochemical Screening

Phytochemical screening of the ethanolic extract was done

Acute Toxicity study

The LD_{50} of the *A. Mellifolium* was reported to be safe till 850-1560 mg/kg/*p.o.* Thus the studies were carried out by using two selected doses of ethanolic extract of *A. Mellifolium* (EEAE)(100mg/kg and 125mg/kg). The dose of plant was selected by hit and trial method. No death and side effects were found at both selected doses of plant.

Experimental Design

Methodology involves one model: (i) Pyloric Ligation-induced Ulcers

The study was comprised of 9 groups and each group comprised of 6 rats.

Group I (Normal Control): Rats were maintained on standard food and water and no treatment was given.

Group II: (Disease Control)

(a) Pylorus-ligation induced ulcers: Overnight fasted rats were subjected to pylorus ligation and post surgical care was given to all animals.

Group III: (Standard drug treated group):

(a) Omeprazole (20 mg/kg) treated pylorus ligated rats: Rats were treated with omeprazole (20 mg/kg/*p.o.*) 30 min before they were subjected to ligation.

Group IV: (Pre-treatment Group)
(a) *A. Mellifolium* (100 mg/kg) treated pylorus-ligated rats: *A. Mellifolium* (100 mg/kg/*p.o.*) was administered 30 minutes prior to pylorus ligation.
(b) *A. Mellifolium* (125 mg/kg) treated pylorus-ligated rats: *A. Mellifolium* (50 mg/kg/*p.o.*) was administered 30 minutes prior to pylorus ligation.

Evaluation of ulcer
The stomachs were harvested, opened along the greater curvature and the mucosa was exposed for macroscopic evaluation. The ulcerated area was assessed and the ulcer index was calculated as the arithmetic mean for each treatment (Suzuki *et al.,* 1976).

Mean Score for evaluation of ulcer index
Ulcer index was determined by the scoring method of Suzuki *et al.* (1976).

Ulcer Protection
The percent protection with each test drug dose was calculated by the formula described by Bhalke *et al.,* (2010).
% Protection = (UI control − UI treated)/(UI control) × 100Where, UI stands for ulcer index.

Determination of pH and Total Acidity
Gastric juice was collected from pylorus ligation control. The collected gastric juice was centrifuged at 1000 rpm for 10 min. The volume of gastric juice was measured. The gastric juice was used for determining pH and total acidity. The pH of gastric juice was measured by using pH meter.1ml of Gastric juice was taken in to a 100 ml conical flask and 2-3 drops of phenolphthalein solution (indicator) was added to the conical flask. Then, the titration was continued until a definite red tinge reappears (Karthik *et al.,* 2010). The volume of alkali added was noted which corresponds to total acidity. Acidity was calculated by using the formula;
Acidity (milli eq/litre/100g) = Volume of NaOH × Normality of NaOH × 100/0.1

Assessment of oxidative stress in tissue

Preparation of Tissue Homogenate
The stomach was weighed and homogenized in chilled phosphate buffer (pH 7.4) at a concentration of 10% (w/v). The homogenate was then centrifuged at 10,000 x for 20 min. The clear supernatant was used for the assays of various parameters.

Estimation of Reduced Glutathione (GSH)
GSH was determined by the method of Jollow *et al.,* 1974. 1ml of homogenate was precipitated with 1ml of 4% sulfosalicilic acid (SSA). The samples were then incubated at 4°C for one hour followed with centrifugation at 1200 g for 20 min at 4°C. The assay mixture contained 0.4 ml supernatant, 2.6 ml sodium phosphate buffer (0.1M, pH 7.4) and 0.2 ml 5, 5'-Dithio-bis (2-nitrobenzoic acid (DTNB) (100 mM) in a total volume of 3.0 ml. Absorbance was studied at 412 nm immediately after the appearance of yellow color on a spectrophotometer.

Estimation of superoxide dismutase (SOD) activity
SOD was estimated in terms of reduced nitroblue tetrazolium (NBT) using method of Wang *et*

al. (1998). The tissue was minced and homogenized in a mixture of 0.1 N sodium hydroxide (NaOH) and 0.1% sodium dodecyl sulphate (SDS) in water containing 40 mg/L of diethylenetriamine pentaacetic acid (DTPA). The mixture was centrifuged at 20,000 g for 20 min and the resultant pellets were suspended in 1.5 ml of pyridine and kept at 80 °C for 1.5 hours to extract formazan, an adduct formed after reaction of reduced NBT with superoxide anions. The mixture was again centrifuged at 10,000 g for 10 min and the absorbance of formazan was determined spectrophotometrically at 540 nm.

Estimation of Lipid Peroxidation

The estimation of thiobarbituric acid reactive substances (TBARs) was determined by the method of Ohkawa *et al*. (1979). The reaction mixture contained 0.1 ml of sample, 0.2 ml of 8.1% SDS, and 1.5 ml of 20% acetic acid solution and 1.5 ml of 0.8% aqueous solution of TBA (Thiobarbituric acid). The pH of 20% acetic acid solution was adjusted with NaOH above pH 3.0. The mixture was finally made up to 4.0 ml with distilled water (DW), and heated at 95°C for 60 min. After cooling under tap water, 1.0 ml of DW and 5.0 ml of the mixture of n-butanol and pyridine (15: 1, v/v) were added, and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm.

Estimation of Nitrite/Nitrate

The estimation of nitrite in the supernatant was determined using a colorimetric assay with the Griess reagent as described by Green *et al*., 1982. Equal volumes of supernatant and the Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDA), 1% sulfanilamide and 2.5% phosphoric acid) were mixed. Then, the mixture was incubated for 10 min at room temperature in the dark, and the absorbance was measured at 540 nm (Green *et al.,* 1982; Kumar and Kumar., 2008).

Estimation of gastric adhesion mucus content

Gastric wall mucus was determined according to the procedure of (Corne *et al*., 1974). The glandular segments from stomachs which had been opened along their greater curvature were removed and weighed. Each segment was transferred immediately to 10 ml of 0.1% w/v Alcian blue solution (in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate pH 5). After immersion for 2 h, excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose, first for 15 and then for 45 min. Dye complexed with the gastric wall mucus was extracted with 10 ml of 0.5 M magnesium chloride ($Mgcl_2$) by shaking intermittently for 1 min at 30 min intervals for 2 h. Four milliliters of blue extract were then shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of net glandular tissue was then calculated.

Assay of Myeloperoxidase activity (MPO)

MPO activity was measured by the method of (Qiu *et al*., 1996). Firstly; the gastric mucosa was scrapped after examination of ulcer index. The scrapings were then homogenized in ice-cold phosphate buffer. Hexadecyltrimethyl ammonium bromide (0.5% HTAB) (Sigma) was added to this phosphate buffer (50 mM, pH 6.0) to release MPO from the primary granules of neutrophils.

Homogenates were then centrifuged and the supernatants were aspirated and mixed with o-dianisidine hydrogen peroxide reagent (Aldrich Chemical Co.) and absorbance at 460 nm was measured with a spectrophotometer. One unit of MPO activity was defined as that degrading 1 mmol of peroxide per minute at 25 per g protein of gastric mucosa.

Assesment of integrity of stomach using Histopathological studies

The stomachwasexcisedandimmediatelyimmersedin10%buffered formalin.Theywerethendehydratedinthegradedconcentrationsofethanol,immersedin xylene, and thenembedded in paraffin. From theparaffin blocks, 4-mmthin sections were cut, and stainingis doneusingwith haematoxylin (0.6%w/v)for15 min followed by counterstaining with eosin (1% w/v) for 2 min. They were then examined using light microscopytoanalyze integrity of stomach, usinganimageanalysisprogram(NIH Scionimageanalyzer).

Estimation of Protein Content

Protein content was estimated by Biuret method using protein estimation kit.

Statistical analysis

All the results were expressed as Mean ± SEM. Data analysis was performed using Graph Pad Prism Version 5.0 software. Statistical comparisons were made between drug treated groups and disease control rats. Data of biochemical parameters were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple range test. The p-value <0.05 was considered to be statistically significant.

Results

The treatment with *A. Mellifolium* (100mg/kg and 125mg/kg) did not produce any significant effect on various parameters performed in the present study. However, when results for treatment with ethanol alone versus no treatment (Normal control) were compared, ethanol caused significant injury to the gastric mucosa and altered the gastric mucosal indicators.

Effect of *A. Mellifolium* on Pylrous Ligation-induced ulcers.

Effect of *A. Mellifolium* on ulcer index in pylorus ligated rats.

It was observed that in pylorus ligated group, the ulcer index was 91.4±8.12 and the maximum no of the ulcers were 4 and 5.

Pretreatment with omeprazole and EEAM (100mg/kg) significant decrease in the ulcer index to 5.5±2.15 and 17.3±2.34 respectively and EEAM (125mg/kg) produce less significant reduction in ulcer index to 69.1±2.34.

Effect of *A. Mellifolium* on volume of gastric fluid in pylorus ligated rats

Pylorus ligation caused marked increase in gastric secretions which was found to be 2.1±0.24 EEAM (100mg/kg) and omeprazole produced significant decrease in gastric secretions to 0.783±0.08, 1.03±0.08 respectively. EEAM (125mg/kg) produced no significant decrease in the gastric fluid volume.

Effect of *A. Mellifolium* on total acidity in pylorus ligated rats.

Level of total acidity was significantly increased in the rats subjected to the pylorus ligation

(101.1±5.43). Pretreatment with EEAM (100mg/kg) showed marked decrease in the total acidity 25.6±3.42. Marked decrease in total acidity was also observed in omeprazole treated rats (15.5±3.41). Lesssignificant decreasein total acidity was observed in rats treated with EEAM (125mg/kg) to72±5.66.

Effect of *A. Mellifolium* on pH in pylorus ligated rats.

Results indicated that pH was reduced in disease control (2.1±0.23). However prior and EEAM (100mg/kg) (3.6±0.05) increased the level of pH. The similar effect was observed in the rats pretreated with omeprazole to 4.3±0.31 but EEAM (125mg/kg) produced no significant decrease in pH.

Effect of *A. Mellifolium* on superoxide dismutase in pylorus ligated rats.

SOD level was significantly reduced in the rats subjected to pylorus ligated rats by 1.74±0.28 in respect to normal control (8.91±0.84). Pretreatment with EEAM (125mg/kg) increased the level of SOD to 4.21±0.45. But the marked increase in the SOD level was observed in rats treated with 100mg/kg of EEAM (6.70± 0.23) and omeprazole (7.48±0.83).

Effect of *A. Mellifolium* on glutathione in pylorus ligtated rats.

Rats subjected to the pylorus ligation showed a marked decrease in GSH level by 0.288±0.033. Pretreatment with EEAM (100mg/kg) and omeprazole significantly increased the GSH level to 0.540±0.041, 0.78±0.009 respectively. EEAM (125mg/kg) produce less significant increase in the GSH level (0.305±0.034).

Effect of *A. Mellifolium* on gastric adhesion mucus content in pylorus ligated rats.

Level of gastric adhesion mucus content was .significantly decreased in disease control (148.1±10.27) in comparison to normal control (210±7.56). Pretreatment with EEAM (100mg/kg) and omeprazole resulted in significant increase in the level of gastric adhesion mucus content 201.3± 6.06 and 211± 5.34 respectively. But less significant increase in gastric adhesion mucus content was noted in rats treated with EEAM (125mg/kg) to 194±3.90.

Effect of *A. Mellifolium* on Myeloperoxidase in pylorus ligated rats.

Level of MPO was markedly increased in the pylorus ligated rats (19.3±1.20) as compared to normal control (12.9±0.85). But this increase in MPO level was significantly decreased by pretreatment with EEAM (100mg/kg) and omeprazole to 17.9±0.53, 14.3±0.90 respectively. However, EEAM (125mg/kg) produce no significant decrease in MPO level.

Effect of *A. Mellifolium* on nitric oxide in pylorus ligated rats.

Rat subjected to the pylorus ligation produced marked decreased in the level of NO in disease control (2.68±0.41) in comparison to normal control (10.05±0.19). Pre-treatment with EEAM (100mg/kg) and omeprazole significantly increases the NO level to 5.08±0.33, 7.86±0.33 respectively. However less significant decrease in NO level was observed in treatment with and EEAM (125mg/kg) to 3.77±0.42 (Table 2).

Effect of *A. Mellifolium* on thiobarbituric acid reactive substances in pylorus ligated rats.

TBARs level was significantly increased in rats subjected to the pylorus ligation (15.90±1.53) in

comparison to normal control (3.92±0.510). Pre treatment with EEAM (100mg/kg) and omeprazole showed significant decrease in the TBARs level to 3.51±0.547, 4.85±0.654 respectively. But a significant decrease in TBARs level was also noted in rats treated with EEAM (125mg/kg) (9.05±0.450) respectively.

Histopathological Evaluation of Gastric Lesion

Histopathological observation of pylorus liagted rat showed extensive submucosal leukocyte infiltration, submucosal haemorrhage, mucosal ulceration and severe haemorrhage. Further, severe edema was too observed in rats administered with ethanol. However treatment with 100mg/kg of EEAMproduced mild mucosal damage resulted in lack of submucosal edema, lack of inflammatory infiltrate and focal mucosal damage. Whereas EEAM (125mg/kg) restore gastric mucosa but not in a significant manner, resulted in moderate Submucosal edema and leukocyte infiltrate (Fig 1).

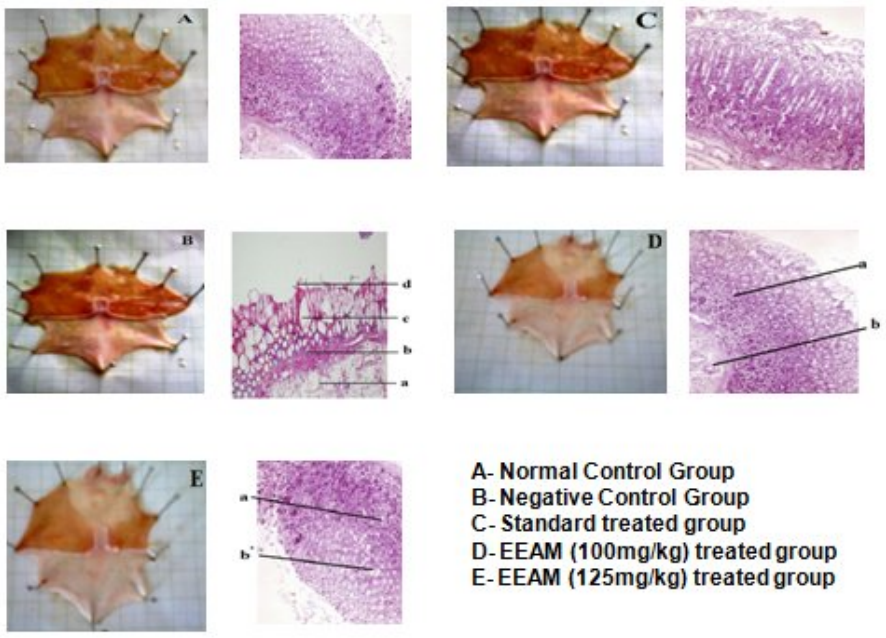

Fig. 1: Histopathological observation of pylorus liagted rat showed extensive (a) submucosal leukocyte infiltration, (b) submucosal haemorrhage, (c) mucosal ulceration and (d) severe haemorrhage

Discussion

Gastric mucosa can withstand exposure to highly concentrated HCL, refluxed bile acids salts, alcohol, and spicy food and with a wide range of temperature and osmolarities (Wallace, 2000). Disruption of the balance between the local release of vasodilator and vasoconstrictor mediators could therefore be involved in the pathogenesis of mucosal injury (Whittle *et al*., 1989; Demir *et*

al., 2003)**.** Further, imbalance between the aggressive and defensive factors contributes damage to the mucosal integrity by decreasing mucosal blood flow, bicarbonate secretion and gastric secretions (Piper and Stiel, 1986; Marhuenda *et al*., 1993). Herbal plants play a vital role in the management of various diseases like Parkinson Diseases (Chen *et al*., 2007), Peptic Ulcer (Goel *et al*., 2002), Diabetes (Modak *et al*., 2007), Neurodegenrative disorders (Kim and Oh, 2012), Alzheimers Diseases (Man *et al*., 2008). *Centella Asiatica* (Abdullah *et al*., 2010), *Aspilia*

Table 1: Effect of EEAM On various gastric parameters in pylorus ligated rats

Parameters / Group	Ulcer index	Total Acidity	Volume of Gastric fluid	pH
Control	91.4±8.12	101.1±5.43	2.1±0.24	2.1±0.23
OmeprazoleTreated group	5.5±2.15***	15.5±3.41***	0.783±0.08***	4.3±0.31***
EEAM(100mg/kg) (125mg/kg)	72.1±5.66***	72±5.66***	1.88±0.17**	2.1±0.34***
EEAM(100mg/kg)	69.1±2.34*	25.6±3.42*	1.03±0.08^{NS}	3.6±0.05^{NS}

Values are expressed as mean±SEM; control group was compared with normal control group. Extract treated group were compared with diseased control. *P>0.05, **p>0.01, ***p>0.001

Table 2:Effect of EEAM On various biochemical parameters in pylorus ligated rats

Parameters / Group	TBARS (nmol/ml)	GSH (µmol/ml)	NO (µmol/ml)	SOD (unit/mg protein)	MPO (µ/protein)	Gastric Adhesion Mucus content (µg wet glandular tissue)
Normal	3.92±0.510	0.786±0.038	10.05±0.19	8.91±0.84	12.9±0.85	210±7.56
Control	15.90±1.53	0.288±0.033	2.68±0.41	1.74±0.28	19.3±1-20	148±10.27
Standard	4.85±0.654***	0.78±0.09***	7.86±0.33***	7.48±0.83**	14.3±0.90***	211.3±6.06***
EEAM(100mg/kg)	9.05±0.456***	0,305±0.034**	3.77±0.42***	4.21±0.45**	17.9±0.53**	201.3±6.06**
EEAM(125mg/kg)	3.92±0.510*	0.540±0.41*	7.86±0.33*	6.70±0.23*	19.0±0.75^{ns}	194±3.90*

Values are expressed as mean±SEM; control group was compared with normal control group. Extract treated group were compared with diseased control. *P>0.05, **p>0.01, ***p>0.001

Africana (Ubaka *et al*., 2010), *Benincasa Hispida* (Shetty *et al*., 2008), *Ficus Arnottiana* (Khan *et al*., 2011), *Morinda Citrifolia* (Srinkanth and Murlidharan, 2009), *Ocimum Sanctum* (Pattanayak *et al*., 2010), *Psidium Guajava* (Das *et al*., 2009) are found to possess antiulcer activity with high efficacy and no side effects (Sravani *et al*., 2011). Various synthetic drugs like H2 blockers and Proton pump inhibitors are employed in the mangment of gastric ulcer but they possess serious adverse effects (Muchandi and Chandrashekhar, 2011). This has been the major stimulus for the development of new antiulcer drugs and anti-inflammatory for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse (Wahida *et al*., 2007). Herbal drugs constitute a major part of therapeutics in all the traditional systems of medicine. Herbal medicine is a triumph of popular therapeutic diversity and is now emerging as an alternative treatment to available synthetic drugs for treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness (Ubaka *et al*., 2010). Many tropical herbs have been scientifically reported to possess potent antiulcer activity. Most of the anti-secretory drugs reduce acid secretion, thus giving immediate symptomatic relief but there are reports of adverse effects and relapse in the long run (Goel *et al*., 2002). On the contrary herbal drugs mostly augment the defensive factors and may be slow in activity but are reliable and safe (Goel *et al*., 2002).

The present study has been designed to explore the Ameliorative and antioxidant effect of *A. Mellifolium* in pylorus-ligation induced ulcers. *A. Mellifolium* has been used traditionally but still no clinical research has been conducted that report its antiulcer activity. *A. Mellifolium Linn* (Hyssop, Family: Lamiaceae), a perennial herb with a long history of medicinal use and its *A. Mellifolium Linn* (Hyssop, Family: Lamiaceae), referred to as "yarrow", have been known for over 3000 years (Mitich L.,1990), and have been used in folk medicine against several ailments such as skin inflammations, spasmodic and gastrointestinal disorders, hepato-biliary complaints (benedek B.,2007). The medicinal properties of *Achillea* are appreciated worldwide and the plants are included in the national *Pharmacopoeias* of some countries like Germany, the Czech Republic and France (Blumenthal M., 2000).Concerning the bioactivity of this plant, recent studies reported antimicrobial, antiphlogistic, hepatoprotective, antispasmodic and calcium antagonist activities of its polar extracts (Stojanović *et al.,* 2005; Yaeesh *et al.,* 2006), and a protective effect of its infusions against H2O2- induced oxidative damage in human erythrocytes and leucocytes (Konyalioglu & Karamenderes, 2005).

Ethanol serves as a most common ulcerogenic agent and has been shown to increase the risk of ulcer in humans and has been evident to produce potent ulceration in rats (Ukwe *et al*., 2010; Ibrahim *et al*., 2012). Ethanol is commonly used for inducing ulcers in experimental rat and leads to intense mucosal damage (Lin *et al*., 2012). Research has reported that binge drinking or long-term drinking can cause acute or chronic gastric mucosal injury (Salih, 2007; Ning *et al*., 2012). Alcohol has been found to rapidly penetrate the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water (Gupta *et al*., 2012).

Pylorus ligation leads to the progression of gastric ulcers by accumulation of gastric juice

in stomach damaging the balance of aggressive factors (Kodati *et al*., 2010). The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is believed to increase gastric acid secretions (Karimulla and Kumar, 2012) Further, in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation aare found to be responsible for induction of ulceration (Devi *et al*., 2012). This contention is supported by the fact that experimental models used in the present study resulted in increased acid secretion observed by increased ulcer index and increased total acidity. Oxidative stress has been reported to play an important role in the progression of ulcer (Tandon *et al*., 2004; Srivastava et al., 2011). Generation of these ROS plays a major role in the development of multiple pathologies, such as gastritis, peptic ulcerations or gastric adenocarcinoma (Chakraborty *et al*., 2012; Uduak *et al*., 2012). Increased oxidative stress results in the generation of ROS that resulted in imbalance in the endogeneous antioxidants and cellular damage in both ethanol and pylorus ligation-induced ulceration (Qader *et al*., 2012; Suleyman *et al*., 2001; Kandhare *et al*., 2011; Alrashdi *et al*., 2012; Kumar *et al*., 2012). Results revealed that in both ethanol and pylorus ligation there is increased in the level of TBARS along with marked attenuation in the level of endogeneous antioxidants (GSH and SOD) which suggests its free radical scavenging property.

Moreover, it has been demonstrated in various studies that excessive production of myeloperoxidase (MPO) that exists in neutrophil leukocyte cells and catalyses the formation of toxic hypochlorous acid (HOCl) from hydrogen peroxide causes cell membrane damage by lipid peroxidation (Dursun *et al*., 2009; Alrashdi *et al*., 2012).There is growing evidence that the major source of ROS is from the activated neutrophils (Pan *et al*., 2008) and neutrophils play an vital role in the development of gastric damage by their aggregation and release of tissue-disrupting substance, such as oxygen free radicals and proteases (Kobayashi *et al*., 2001; Lin *et al*., 2012).The neutrophil infiltration into the gastric mucosal tissues is assessed by MPO as well as NOS (Coskun *et al*., 1996; Takeuchi *et al*., 1998). In the present study, we observed a significant increase in MPO activity in the stomach following ethanol administration and pylorus ligation, which confirmed the infiltration and activation of neutrophils in the gastric mucosa produced by both ethanol and pylorus ligation.

Studies suggested that both ethanol and pylorous ligation caused severe micro vascular injury, namely disruption of vascular endothelium resulting in increased vascular permeabiltity, edema formation and epithelium lifting (Vidya *et al*., 2012). Further, Ethanol has been reported to produce necrotic lesions in gastric mucosa by its direct toxic effect, reducing the secretions of bi-carbonates and production of mucus (Alrashdi *et al*., 2012). Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and narcotic aspects of tissue injury (Swapna *et al*., 2011). The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury (Hernandez-Rincon *et al*., 2003) and results in cell death along with exfoliation in the surface epithelium. This contention is supported by results obtained from histopathological studies of stomach of both ethanol and pylorus ligated rats. Nitric oxide plays an important role in the host defense and inflammatory response (Pan *et al*., 2005). It also plays an important role in the mechanism of gastric mucosal protection and injury induced by pressure, ethanol, stress

and endotoxins (Pique *et al*., 1989, 1992; Tepperman and Soper, 1994; Qiu *et al*., 1996; Nishida *et al*., 1997; Zhang *et al*., 2000; Pan *et al*., 2005). During the past decade, the endothelial-derive relaxation factor nitric oxide (NO) has been recognized as one of the important mediators for the regulation of gastric mucosal microcirculation, repair, and integrity (Oda *et al*., 1998; Catalayud *et al*., 2001; Ma *et al*., 2001) Inhibition of NO synthesis has been shown to produce acute gastric mucosal damage (Martin *et al*., 2001; Tariq *et al*., 2007) whereas enhancement of NO synthesis exerts gastro protective effects. NO expressed in gastric mucosa and showed to increase gastric blood flow, mucus secretion and reduce neutrophil adhesion (Nabavizadeh *et al*., 2011; Lin *et al*., 2012). Ethanol and pylorus ligation leads to the marked decreased in the NO level which results in the increased neutrophil adhesion and decrease blood flow (Alrashdi *et al*., 2012). Gastric adhesion mucus content acts as a barrier against various offensive factors (Zalwesky and Moody, 1979; Flemstrongand Garner, 1982; Marhuenda *et al.,* 1993; Kalra *et al.,* 2011; Geetha and Sarnaya, 2012). According to Hiruma-Lima *et al*. (2006) gastric mucus is a viscous, elastic, adherent and transparent gel formed by water and glycoprotein's covering the entire gastrointestinal mucosa. Reports indicated that that the protective properties of the mucus barrier depend not only on its gel-like structure, but are also related to the amount or thickness of the layer covering the mucosal surface. Mucus protects the gastric mucosa against various irritants (Borra *et al*., 2011). Moreover, mucus is capable of acting as antioxidant and thus can reduce mucosal damage mediated by oxygen free radicals (Reptto and Llseuy, 2002). Our study too revealed that in both experimental models there is sharp decrease in the level of gastric mucus content.

Literature survey indicated that*A. Mellifolium* is mainly used for antispasmodic, stomachic, antifungal and cough treatments. In the present study we have investigated the role of *A. Mellifolium* in the treatment of ulcers. The Pharmacological treatment with *A. Mellifolium* implicated that it significantly attenuated the level of gastric secretion, ulcer index and total acidity which indicated that plant may possess antisecretory property. Further, it has been found that treatment with *A. Mellifolium* (100mg/kg and 125mg/kg) produced significant decrease in TBARs level and marked attenuation in the level of natural endogeneous antioxidants GSH and SOD. Moreover, treatment with *A. Mellifolium* (100mg/kg and 125mg/kg) attenuated the level of MPO, marker of lipid peroxidation and significantly increased the level of NO that plays an important role in ulcer healing. In addition, a significant increase was found in the mucus content when the rats subjected to pylorus ligation were treated with *A. Mellifolium* extract which showed the gastroprotective property of the plant. In addition, Histopathological results of our studies revealed that treatment with *A. Mellifolium* (100mg/kg) results in the maintainace of mucosal integrity and mild mucosal ulceration.

Thus, it may be concluded that *A. Mellifolium* may possessed gastroprotective property determined by increase in the level of mucus and marked attenuation in the level of MPO. Further, it may possess antioxidant activity as it significantly attenuated the level of TBARS and increase the level of GSH and SOD. Moreover, the extract of the plant was also found to be beneficial in maintain the level of NO.

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