Gastro and Cytoprotective activity of Indian Medicinal Plants against Ethanol Induced Gastric Ulcer in Rats

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Abstract- *The antiulcer activity of the poly herbal formulation (composed of the leaf extracts from Lantana camara, Annona muricata, Kalanchoe pinnata) was evaluated in ethanol induced ulcer model in rats. The extract at dose of 1000mg/kg produced significant inhibition of gastric lesion induced by above mentioned method. The extract reduced ulcerative lesion, gastric volume, free and total acidity and pH of gastric juice in the model. The result obtained suggesting that extract possesses significant anti-ulcer activity.*

Keywords- Antiulcer, gastric lesion, Lantana camara, Annona muricata, Kalanchoe pinnata, free acidity, total acidity, ulcer index, gastric juice.

I. INTRODUCTION

Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by action of the gastric juice. It is a chronic and recurrent disease, and is the most predominant of the gastrointestinal diseases (Golan DE, etal., 2008). It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors (Falcao HS, etal., 2008). Gastric ulcer is among the most serious diseases in the world. The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid– pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermic growth factors (Repetto MG and Llesuy SF. 2002). Some other factors, such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by Helicobacter pylori, may be responsible for the development of peptic ulcer (Peckenpaugh NJ and Poleman CM. 1997).

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries. Peptic ulcers are illnesses that affect a considerable number of people in the world (Falk GW. 2001)

Nature has provided a complete storehouse of remedies to cure ailments of mankind,there is a widespread belief that green medicines are healthier, though the recovery of disease by plant medicine is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and effective for antibiotic resistant microorganisms (Perumalsamy et al., 2008). India is well known for its rich traditional system of medicine, besides a vast reservoir of living traditions of ethno medicine.

As a result, more and more synthetic drugs are introduced and offering newer options for treatment of peptic ulcer. Because of several side effects of synthetic medicines, there is new thought of better natural alternative for the treatment of peptic ulcer. Medicinal plants are frequently used in traditional medicines to treat various diseases in different parts of the world.

Medicinal plants represent an important source of medically important compounds. Since ancient time, medicinal plants are used to cure several types of health problems. Systemic analysis of these plants provides a variety of bioactive molecules for the development of newer pharmaceutical products. Recently, there is a growing interest in the pharmacological evaluation of various plants used in different traditional system of medicine. In last few decades, many of traditionally known plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer activity, anti-inflammatory activity, antidiabetic activity, anthelmintic, antibacterial activity, antifungal activity, hepatoprotective activity, antioxidant activity, larvicidal activity etc (Rajkumar V et al. 2009., Kumar SV, Sankar P and Varatharajan R. 2009., Sabu MC and Kuttan R. 2002.,). Lantana camara Linn. is a flowering ornamental plant belonging to family Verbenaceae. L. camara is also known as Lantana, Wild Sage, Surinam Tea Plant, Spanish flag and West Indian lantana. L. camara is a well-known medicinal plant in traditional medicinal system and recent scientific studies have emphasized the possible use of L. camara in modern medicine.

Lantan camara introduced in India as an ornamental plant but entirely naturalized and found throughout India.

However, it is listed as one of the significant medicinal plants of the world (Ross. 1999).The plant Lantana camara (Verbanaceae), generally known as wild or red sage is the most widespread species of this genus and it is a woody straggling plant with various flower colors, red, pink, white, yellow and violet. It is an ever green strong smelling shrub, with stout recurred prickles, leaves opposite, ovate, acute or sub- acute, crenate -serrate, scab rid on both side (Thamotharan G. 2010).

Scientific classification

Kingdom: Plantae
Order : Lamiales
Family : Verbenacea
Genus : Lantana
Species : camara

Morphology of L. camara is reported in Figure 1. L. camara is a low erect or subscandent vigorous shrub with tetrangular stem, stout recurved pickles and a strong odour of black currents. Plant grows up to 1 to 3 meters and it can spread to 2.5 meter in width. Leaves are ovate or ovate oblong, acute or sub acute crenate serrate, rugose above, scabrid on both sides. The leaves are 3-8 cm long by 3-6 cm wide and green in colour. Leaves and stem are covered with rough hairs. Small flower held in clusters (called umbels). Colour usually orange, sometime varying from white to red in various shades and the flower usually change colours as they ages. Flowers are having a yellow throat, in axillary head almost throughout the year. The calyx is small, corolla tube slender, the limb spreading 6 to 7 mm wide and divided in to unequal lobes. Stemen four in two pairs, included and ovary two celled, two ovuled. Inflorescences are produced in pairs in the axils of opposite leaves. Inflorescences are compact, dome shaped 2-3 cm across and contain 20-40 sessile flowers. Root system is very strong and it gives out new fresh shoots even after repeated cuttings (Sastri BN. 1962).

Annona muricata L. belongs to the family of Annonaceae has a widespread pantropical distribution and has been pridely known as corossol. It is a widespread small tree and has its native in Central America (Alassane Wele, Yanjun Zhang, 2004). Intensive chemical investigations of the leaves and seeds of this species have resulted in the isolation of a great number of acetogenins. The isolated compounds display some of the interesting biological or the pharmacological activities, such as antitumoral, cytotoxicity, antiparasitic and pesticidal properties. Roots of these species are used in traditional medicine due to their antiparasitical and pesticidal properties (Christophe Gleye, 1997).

Scientific classification

Kingdom: Plantae-Plants
Class: Magnoliopsida
Order: Magnoliales
Family: Annonaceae
Genus: Annona
Species: muricata

The genus name ‘Annona’ is from the Latin word ‘anon’, meaning ‘yearly produce’, referring to the fruit production habits of the various species in this genus. Annona muricata is a slender, evergreen tree, 5-10 m in height and 15 cm in diameter; trunk straight; bark smooth, dull grey or grey-brown, rough and fissured with age; inner bark pinkish and tasteless; branches at first ascending with the crown forming an inverted cone, later spreading; crown at maturity spherical due to lack of apical dominance; twigs brown or grey, bearing minute raised dots (lenticels); root system extensive and superficial, spreading beyond the diameter of the crown although shallow rooted; juvenile plants have a taproot that is eventually lost. Leaves alternate, 7.6-15.2 cm long, 2.5-7.6 cm wide, leathery, obovate to elliptic, glossy on top, glabrous on underside, simple; stipules absent; blade oblanceolate, green on top, paler and dull on under side with fine lateral nerves; a strong, pungent odour; petioles short, 3-10 mm long (Anon. 1986).

The knowledge of traditional medicine and medicinal plants and their study of scientific chemical principles may lead to the discovery of newer and cheaper drugs. Kalanchoe pinnata (Lam., syn. Bryophyllum pinnatum, B. calycinum; Local name: Pathorkuchi, Coughpatha; English name: Air plant; Family: Crassulaceae) is an herb found ubiquitously in Bangladesh. It has tall hollow stems, fleshy dark green leaves that are distinctly scalloped and trimmed in red, and bell-like pendulous flowers (Ghani A. 2003). Kalanchoe pinnata (K. pinnata) has become naturalized in temperate regions of Asia, Australia, New Zealand, West Indies, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia, and Hawaii. It is also widely distributed in the Philippines, where it is known as katakataka or katakataka which means astonishing or remarkable (Ghani A. 2003, Descoings B. 2003). The leaves of K. pinnata have a variety of uses in the traditional system of medicine in Bangladesh. They are eaten for diabetes, diuresis, dissolving kinney stones, respiratory tract infections, as well as applied to wounds, boils, and insect bites (Ghani A. 2003). It is useful for preventing alcoholic, viral and toxic liver damages. The aqueous extract of this plant have shown anti-inflammatory, anti-diabetic, anti-tumor and cutaneous leishmanicidal activities (Sidhartha PA, 1990, Supratman

UT, Fujita K, 2000, Torres-Santos ECS, 2003, Muzitano MF, 2009).

Scientific classification

Kingdom: Plantae-Plants
Class: Magnoliopsida
Order: Saxifragales
Family: Crassulaceae stonecrop family
Genus: Kalanchoe
Species: pinnata

Kalanchoe pinnata (Family: Crassulaceae) is an important plant which has many traditional medicinal uses. Kalanchoe pinnata (Family: Crassulaceae) is an erect, succulent, perennial shrub that grows about 1.5 m tall and reproduces through seeds and also vegetatively from leaf bubils. It has a tall hollow stems, freshly dark green leaves that are distinctively scalloped and trimmed in red and dark bell-like pendulous flowers. This plant can easily be propagated through stems or leaf cutting. It is an introduced ornamental plant that is now growing as a weed around plantation crop. K. pinnata is used in ethnomedicine for the treatment of earache, burns, abscesses, ulcers, insect bites, whitlow, diarrhoea and cithiasis (Okwu and Nnamdi. 2011). In traditional medicine, Kalanchoe species have been used to treat ailments such as infections, rheumatism, and inflammation (Nayak et al., 2010) and have immunosuppressive effect as well (McKenzie and Dunster. 1986).

II. MATERIALS AND METHODS

Collection And Extraction Of The Plant

The leaves of L.camara, A.muricata and K.pinnatum were collected around Vellore district. After washing the plant with running water, the leaves were separated and dried in shade for 20 days at room temperature. After shade drying, the leaves were grinded through blender and converted into coarse of powder. The powder was extracted by continuous hot extraction using the Soxhlet apparatus. The extracts were collected and preserved in a desiccator until used for further studies.

Test animal

Adult healthy wistar rats weighting 150 g were used and kept in the animal house. The animals were kept in plastic cages (34 × 47 × 18 cm3) at animal house, in an air conditioned environment with four rats in each cage and maintained at room temperature of (25 ± 2) _C with relative humidity (60% ± 10%) under 12 h night and light cycle. The animals used for the experiment were approved by animal ethics committee.

Preparation and Dose of the Test Drug

The dose of the test drug was calculated by the method of Miller and Tainter (1944), found to be 1000mg/kg the dose of the extract was calculated with reference, the aqueous extract of the drug was used in the dose of 150mg/kg. Standard drug, Rabeprazole (Manufactured in India by Cipla Laboratories Ltd.) was used in the dose of 20mg/kg.

Phytochemical analysis

The preliminary phytochemical analysis of L.camara, A.muricata, K.pinnata leaves aqueous extract was carried out for carbohydrate, saponins, flavonoids, triterpenoids, tanins and alkaloids.

Acute Toxicity Study

The oral acute toxicity study of aqueous extract of L.camara, A.muricata, K.pinnatum Were evaluated according to Organization for Economic Cooperation and Development (OECD) guideline 420 on wistar rats, where the limit test dose of 1000 mg/kg was used. All the animals were kept at overnight fasting before to every experiment with free excess to water. The test drug was administered and observed for 14 days to determine urea, creatinine, SGOT, SGPT level.

EXPERIMENTAL DESIGN

The rats were randomly divided into 6 groups, of 4 rats each as follows:

Group-I: Control group animals received no treatment.
Group-II: animals received 20mg/kg body weight of Ethanol (Negative control).
Group-III: animals received 1000 mg/kg body weight of freshly prepared L.camara.
Group-IV: animals received 1000 mg/kg bodyweight of freshly prepared A.muricata.
Group-V: animals received 1000 mg/kg body weight of freshly prepared K.pinnatum.
Group-VI: animals received 20mg/kg body weight of Rabeprazole.

All treatments were administered orally for 11 days. Score of mucosal damage were microscopically observed.

Histological observation

In the 11th day, after 24 h fasting the animals were sacrificed and stomach of each animal was opened along the greater curvature. Specimens of the gastric tissue were fixed in 10% buffered formalin and were processed in the paraffin tissue-processing machine. Sections of the stomach were sectioned at 5µm and stained with hematoxylin and eosin for histological evaluation (Hajrezaie M. 2012). Paraffin sections were stained with toluidine blue. The effect of drugs was evaluated through assessment of inflammatory and necrotic changes in the mucosal tissue.

Ethanol-induced Gastric Ulceration and Its Protection Studies

Before ulcer induction animals of both control and experimental groups kept separately in standard controlled conditions were fasted for 24 h with free access to water. Acute gastric ulcers were induced by oral administration of ethanol at a dose of 20 mg/ kg body weight and rats were sacrificed after 4 h of ethanol treatment. The control group received the vehicle only, whereas the experimental group received ethanol for gastric ulceration. After 4 h, the animals were sacrificed, and gastric lesions in the fundic stomach were scored and expressed as ulcer index. L.camara, A.muricata, K.pinnata leaves aqueous extract was administered orally 30 min prior to ethanol treatment to see the gastroprotective effect. Rabeprazole were administered orally at a dose of 20 mg/ kg body weight respectively.

Assessment of gross mucosal damage

The lesion in the glandular portion was examined under a 10 x magnifying glass and length was measured using a divider and scale and gastric lesion was scored as follows:

0 - Normal colored stomach,
0.5 - Red coloration,
1- Spot ulceration,
1.5 - Hemorrhagic streak,
2 - ulcers
3- Perforations

Ulcer index of each animal was calculated by adding the values and their mean values were determined and percentage inhibition was calculated (P. Malairajan, G. Gopalakrishnan. 2007).

$$\% \text{ Protection} = \frac{\text{Ulcer index Control - Ulcer index Test}}{\text{Ulcer index Control}} \times 100$$

Determination of pH and volume of gastric juice

Gastric juice (1 mL) was diluted with 1 mL distilled water and was measured using a pH meter and the volume of gastric juice also measured by measuring tubes.

Free and Total Acidity

Free and total acidity were determined by titrating with 0.01 N NaOH using Topfer's reagent and phenolphthalein as indicator. The free and total acidity were expressed as µ_equiv/100 g.

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1\ \text{N}} \times 100$$

III. RESULTS

Preliminary phytochemical screening

The phytochemical screening of the plant extract revealed the presence of various bioactive costituents like alkaloids, flavonoids, saponins and tanins.

Acute Toxicity Study

The oral acute toxicity study of aqueous extract of L.camara, A.muricata, K.pinnatum Were evaluated according to Organization for Economic Cooperation and Development (OECD) guideline 420 on wistar rats, where the limit test dose of 1000 mg/kg was used.
No mortality observed for 14days.

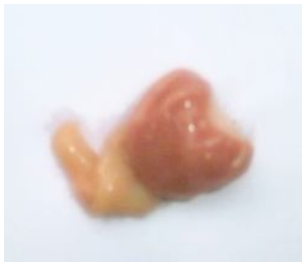
Ethanol Induced Ulcers in Rats

In the present study the anti-ulcer activity of leaves of L.camara, A.muricata, K.pinnatum. Revealed that the minimum ulcer index was observed with Rabeprazole.

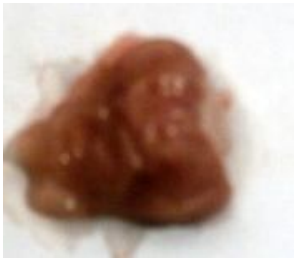
Table 1. Effect of L.camara, A.muricata, K.pinnatum leaves aqueous extract gastric juice volume, pH, total acidity, free acidity, total ulcer index and ulcer protection.

Group	Gastricjuice volume in ml	Gastricjuice pH	Free acidity (mEq/dl)	Total acidity (mEq/dl)	TotalUlcer Index	Ulcer protection(%)
Control	3.97±0.20	3.1±0.30	49.6±0.10	35.2±0.30	0.01±0.00	99.7
Disease control	1.65±0.12	1.52±0.12	96.9±1.12	88.2±0.37	3.82±0.05	12.8
L.camara 150mg/kg	1.8±0.14	2.07±0.17	58.32±1.46	54.32±0.59	2.32±0.02	42.5
A.muricata 150mg/kg	2.05±0.11	2.2±0.19	54.47±0.57	50.1±0.46	1.72±0.09	57.7
K. pinnata 150mg/kg	2.67±0.17	2.65±0.07	52.87±0.20	42.02±0.74	1.27±0.07	68.5
Rabeprazole 20mg/kg	2.82±0.05	2.85±0.07	51.68±0.08	37.95±0.34	0.62±0.08	84.7

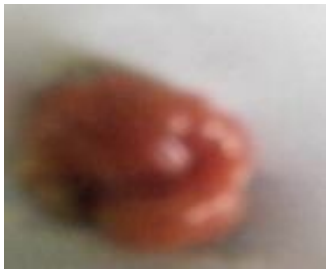
Values are expressed as mean ± SEM. P >0.05 when compared to normal control group by Statistical analysis by One-way ANOVA followed by Dunnett's method.



a. Normal control



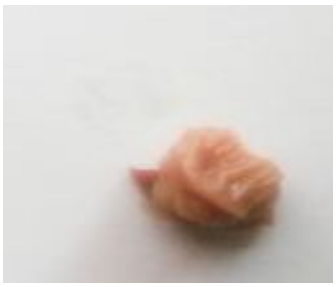
b. Experimental Control



c. L.camara 150mg/kg

d. A.muricata 150mg/kg

e. K.pinnata 150mg/kg

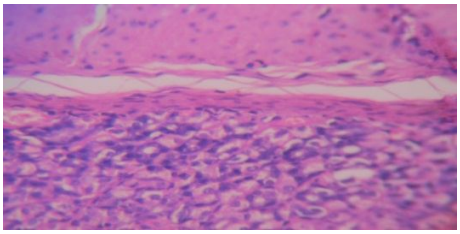


f. Rabeprazole 20mg/kg

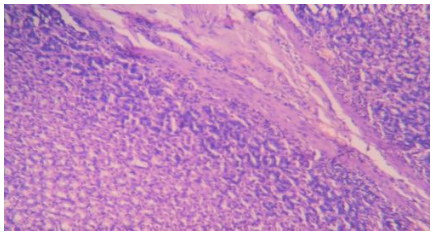
Figure 1. Morphological Features of Stomach in Ethanol Induced Ulcer

Morphological study of stomach

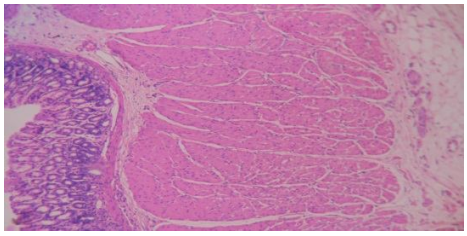
In normal group stomach integrity was maintained and appeared normal. In control group severe bleeding, perforation, spot ulcer were observed but, in standard group and extract treated groups, animal showed less ulceration and stomach integrity was maintained.



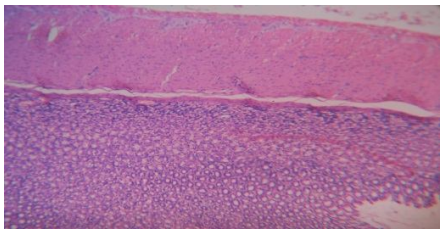
a. Normal control



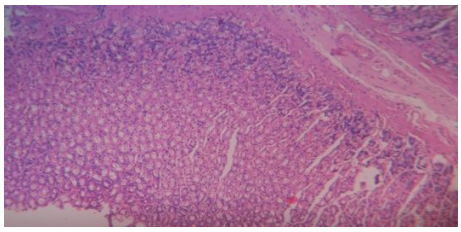
b. Experimental Control



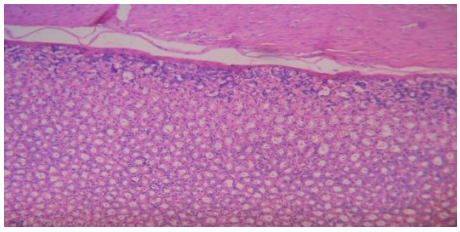
c. L.camara 150mg/kg



d. A.muricata 150mg/kg



e. K.pinnata 150mg/kg



f. Rabeprazole 20mg/kg

Figure 2. Histology of Stomach in Indomethacin induced Ulcer

Histopathological study

Histopathological examination of gastric mucosa in the normal control group showed intact gastric mucosa and continuous epithelial surface. Experimental control revealed mucosal ulceration. In L.camara (1000mg/kg) group, superficial erosions and few ulcers accompanied with mild

inflammatory was observed. In A.muricata (1000mg/kg) group, slight ulcer with inflammatory infiltrate and congestion in few areas was observed. In K.pinnata (1000mg/kg) group, section revealed intact mucosa with no inflammation. In Rabeprazole (20mg/kg) group, showed intact gastric mucosa without any inflammatory.

IV. DISCUSSION

Gastric ulcer result of an imbalance between aggressive and defensive factors of the gastric mucosa (Ateufack G, et al., 2006) factors. Gastric ulcer is among the most serious diseases in the world. The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid–pepsin secretion, parietal cell, mucosal disease barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermic growth factors. Some other factors, such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by Helicobacter pylori, may be responsible for the development of peptic ulcer (Spiro HM. 1991).

To consolidate this balance, different therapeutic agents including medicinal plants are used to reduce gastric acid secretion or enhance mucosal defense mechanisms through increased mucus production (Salena BJ, Hunt RH. 2005).

Drinking absolute ethanol creates hemorrhagic wounds, mucosal friability, and extensive submucosal edema and causes injury to the epithelial cells of the gastric layer (Gornall AG, et al., 1949) Drinking ethanol also decreases the protein concentration due to epithelial cell devastation (Abdulla M, Fard A, Harita H. 2011).

Ethanol causes necrotic lesions of the gastric mucosa in a multifactorial way. It can reach the mucosa by disruption of the mucus-bicarbonate barrier and cause cell rupture in the wall of blood vessels [Mincis et al., 1995]. These effects are probably due to biological actions, such as of lipid peroxidation, formation of free radicals, intracellular oxidative stress, changes in permeability and depolarization of the mitochondrial membrane prior to cell death [Repetto and Llesuy, 2002; Bagchi et al., 1998; Hirokawa et al., 1998]. According to Mizui and Douteuchi [1983], oral administration of HCl/ethanol produces necrotizing lesions in the gastric mucosa, and the lesions are more severe than those caused by absolute ethanol, mainly due to a reduction in the protective layer of mucus and an increase in peptic acid secretion. Like ethanol, ethanol/HCl causes oxidative stress, increases the release of histamine and pepsin, and reduces the levels of DNA, RNA and proteins in the tissue, which results in tissue injury [Batista et al., 2004].

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

Flavonoids are implicated in the protection of the gastric mucosa from necrotizing substances (Kelly Samara de Lira Mota. et al., 2009) and flavonoids are highly useful in the therapy of acute and chronic gastric ulceration (Gopinathan S, Naveenraj D. 2013). Flavonoids have been reported as anti-ulcer agents (Lewis DA, et al., 1999. Mohan Kumar M, et al., 2006. Maury PK, Jain SK. et al., 2012). The antiulcer and gastroprotective effects of quercetin and its glucosides (Martin et al., 1998 and Kahraman et al., 2003) the antioxidant mechanisms involved in gastroprotective effects of flavonoids in ethanol induced ulcerative rats. Antiulcer activity of flavonoids by stimulating Platelet Activator Factor (PAF) in acid– ethanol induced ulcerative animal model (Izzo et al., 1994). Effects of quercetin and other flavonoids on the reserpine induced ulcerogenic mice (Barnaulov et al., 1982. Motilva et al., 1992) recorded the effects of naringenin and quercetin on the acetic acid induced ulcerogenic rats.

Oral administration of RABI (Rabeprazole) significantly reduced ulcer index, gastric juice free and total acidity and pepcin activity. However, the drug has not produced any significant quantitative change in the mucin content. Rabeprazole was reported to significantly increase the production of mucin (a defense factor) in rats. It prevented or reduced the size of gastric ulcers. rabeprazole caused dose-dependent inhibiti Rabeprazole is an inhibitor of the gastric proton pump. It causes dose-dependent inhibition of acid secretion and has a more rapid on of indomethacin-induced ulceration. R(+)-rabeprazole appears to be the major isomer having anti-ulcer activity (Cao H, Wang M, Jia J, 2004).

Phytochemical analysis on the leaves aqueous extract gave positive results for flavonoids, alkaloids, saponins, carbohydrate, tanins and triterpens. The obtained results strongly suggest that flavonoids and alkaloids are the major components of the extract and therefore some of the pharmacological effects could be attributed.

The anti-ulcer activity of the leaves aqueous extract of L.camara, A.muricata, K.pinnatum was evaluated against gastric lesions induced by ethanol.

Treatment with L.camara, A.muricata, K.pinnatum protected the gastric mucosa from damage by increasing the mucin content significantly. Apparently, the free radicals scavenging property of L.camara, A.muricata, K.pinnatum might contribute in protecting the oxidative damage to gastric mucosa.

V. CONCLUSION

Herbal products are well thought-out to be symbols of safeguard in comparison to the synthetic product that are regarded as unsafe to human life and environment. While herbs had been priced for their medicinal significance. The three plants extracts and anti- ulcer drugs compared. Among these, the anti-ulcer drug Rabeprazole and K.pinnatum were more effective than the L.camara, A.muricata.

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