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Original Research Article

EVALUATION OF *ANANAS COMOSUS* FRUIT FOR ANTIULCER POTENTIALS ON EXPERIMENTAL ANIMALS

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Abstract: *Ananas comosus*(L.) Merr., commonly known as pineapple popular fruits across the glove and also popular folk medicine of India, especially of North-East India for the treatment of organ toxicity. To justify the scientific basis in traditional uses as gastro-protective agent, the aqueous (AEAC) and ethanol (EEAC) extracts of *A. comosus* ripe fruits was evaluated for Antiulcer activity using ethanol induced ulcer and Pylorus ligation model in albino rats. The extracts of ripe fruits of *Ananas comosus* shows significant (**p<0.01 and***P<0.001) ulcer-protective activity in dose dependent manner. The ulcer index was significantly reduces in EEAC (**p<0.01) and AEAC (***P<0.001) treated groups when compared with ulcer control group. The pH, free acidity & total acidity level were increased in ulcer control animals when compared with normal control animals and in Pylorus ligation model elevated pH, free acidity & total acidity levels were significantly reduced in EEAC (***p<0.01) and AEAC (***P<0.001) treated groups when compared with ulcer control group. The aqueous extracts were found to be most potent. The published literature shows the presence of tannins, triterpene and flavonoids in aqueous and ethanolic extracts. These observations established the ulcer-protective effect of *A. comosus* and justified the traditional claim. The gastro-protective activities may be attributed to the presence of flavonoids and tannins.

Key Words: *Ananas comosus, Anti-Ulcer,* flavonoids and tannins.

Introduction*:* Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. People worldwide have been using herbal medicine for the treatment, control and management of a variety of ailments since prehistoric times. There is ample archeological evidence to support the fact that primitive man used plant and herbs for medicinal purposes. For instance, pollen analysis of numerous plants found in the grave the Neanderthal man buried 60 000 years ago in Iraq, indicated that the plants buried with the

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corpse were all of medical value. In another example, medicinal herbs found in the personal belongings of the "Ice man" whose body was frozen in the Swiss Alps for more than 5,300 years, are thought to have been used to treat the parasites found in his intestines[1]. Although the direct use of plant extracts in developed countries continued to decrease in the late nineteenth and early twentieth centuries, medicinal plants still play a key role in health care system of many parts of the world. According to World Health Organization 60% of the world's population depend on traditional medicine, and 80% of the population in developing countries depend almost entirely on traditional medical practices. In particular, herbal medicine for their primary health care needs. The long tradition of herbal medicine continues to the present day in China, India and many other countries in Africa and South America. In many village marketplaces of these countries, medicinal herbs are sold alongside vegetables and other wares. Practitioners of herbal medicines in developing countries often undergo a rigorous and extended training to learn the names, uses and preparation of native plants[2]. The medicinal and pharmacological activities of medicinal plants are often attributed to the presence of the so called secondary plant metabolites. Unlike the ubiquitous macromolecules of primary metabolism (e.g. monosaccharide's, polysaccharides, amino acids, proteins, nucleic acids, lipids) which are present in all plants, secondary metabolites with medicinal properties are found only in a few species of plants. Some of these secondary metabolites serve as defensive compounds against herbivores and pathogens. Others function in mechanical support, in attracting pollinators and fruit dispersers, in absorbing harmful ultraviolet radiation, or reducing the growth of nearby competing plants. Secondary plant metabolites with reported medicinal properties include but not limited to polysaccharides, waxes and fatty acids, alkaloids, terpenoids, phenolics (simple phenolics and flavonoids) and glycosides and their derivatives. Some of these secondary plant metabolites are briefly discussed below[3].

Ananas comosus(L.) Merr., commonly known as pineapple popular fruits across the glove and also popular folk medicine of India, especially of North-East India for the treatment of organ toxicity. A perennial long leaved herb grows up to 70cm in height. Leaves numerous compactly and spirally arranged on main stem, liner lanceolate, acuminate, stout with curved spines on margins. Inflorescence head, seen on the apex, small reddish and ovate fruits composite, juicy, bears a tuff of leaves on the tip. Both the root and fruit are sometimes eaten or applied topically as an anti-inflammatory and as a proteolytic agent. It is traditionally used as an antihelminthic agent in the Philippin[4, 5]. The plant reported for its anthelmintic[6], antibacterial[7], antioxidant[7, 8, 9], antidiabetic[10], anti-depression[11], anti-inflammatory[12], analgesic[12] anticancer[13]and wond healing activity[14]. The plant contains β-anthocyanin, catechin and iso-catechin rather than from Vitamin C, E, Terpenoids, Flavonoids, Saponins, Tannins, alkaloids, anthaquinines, sterol, charbohydrates, oils and resigns [15]. The present investigation was aimed to justify the pharmacological basis in traditional use of *A. comosus* as anti-ulcer agent in India and explore action of bioactive component (s) in physiological mechanism of gastro-protection.

Methodology

Collection & Authentication of Plant Material: The ripe fruits of *Ananas comosus* (pineapple) were collected from the villages of Tripura in the month of august and after collection, the fruits were cut into small pieces and dried under the shadow around 1 month at room temperature then subjected to size reduction to a coarse powder with the help of mixer grinder. The plant is authenticated by Dr. B. K. Datta, Professor of Botany, Plant Taxonomy & Biodiversity Laboratory, Department of Botany, Tripura University (A Central University), Suryamaninagar -799022. Tripura, India.

Preparation of Different Extracts: The fruits of *Ananas comosus* were extracted first with

petroleum ether for 18 h. by soxhlet extraction method for defatting and removing waxy substances. The extract was transferred into the previously weighed empty china dish and evaporated to a thick paste on the water bath, maintained at 50^{o}C to get petroleum ether extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated each time before extracting with next solvent. The marc has dried in hot air oven below 50^{o}C to remove the solvent and same marc used for successive extraction with chloroform, ethanolic as solvent one after another in similar manner and each extract have concentrated by distilling off the solvent and then evaporated to dryness on rotator flash evaporator. The petroleum ether, chloroform and ethanol extracts have obtained for the further study[16].

The aqueous extract was prepared by taking the powdered material of fruits of *A. comosus* in a round bottom flask (2000 ml) and macerated with distilled water with 10 ml of ethanol (as preservative) for 24 h with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 50^{o}C.

These extracts were stored in airtight containers in a refrigerator below 10^{o}C. The extracts were examined for their color and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Experimental animals: Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 18-25 g were procured from Sri. Venkateswara Enterprises, Bengaluru for experimental purpose and the animals were acclimatized for 7 days under standard husbandry condition as:

Room temperature - 26 ± 2^{0} C
Relative humidity - 45-55%
Light/ dark cycle - 12:12

Method of determination of acute toxicity: The acute toxicity of extracts of Fruits of *Ananas comosus* was determined in albino mice of either sex weighing between 18-22 g those maintained under standard husbandry conditions. The animals were fasted 3 h prior to the experiment and "up and down" (OECD Guideline No. 420) method of CPCSEA was adopted for toxicity studies. Animals were administered with single dose of extracts and observed for its mortality during 48 h study period (short term) toxicity. Based on the short-term toxicity profile of the extracts the doses of the next animals were determined as per as OECD Guidelines No: 420. All the animals were observed 14 days with special reference[17].

Anti Ulcer Activity

Ethanol induced anti-ulcer model: The incidence of ethanol induced ulcer is predominant in the glandular part of stomach is reported to stimulate the formation of leukotrienes C (LTC), mast cell secreting products and relative oxygen species resulting in the damage of rat gastric mucosa.Albino rats of either sex weighing between (150-200 gms) were divided into 6 groups of 6 animals in each group.

Group A : Control group (vehicle)
Group B : Standard Lansoprazole (8mg/kg P.O.)
Group C : EEAC (200ng/kg P.O.)
Group D : EEAC (400mg/kg P.O.)
Group E : AEAC (200mg/kg P.O.)
Group F : AEAC (400mg/kg P.O.)

Experimental Procedure:

The animals are fasted for 24 hr. with free access to water. Animals were given different extracts of *Ananas co*mosus Linn. mentioned above of 200 mg & 400 mg/kg or Lansoprazole (8mg/kg). 1 hr later 1 ml of 99.80 % alcohol was administered P.O. to each animal. Animals were sacrificed after 1 hour of alcohol administration, stomachs were isolated and cut open along the greater curvature and pinned on a soft board. The ulcer index was measured with the help of hand lens (10X). mean ulcer score for each animal is expressed as ulcer index. The results are compiled in table no 23, fig no 10. Score the ulcers as below[18] –

0 = Normal coloured stomach.
0.5 = Red colouration.
1 = Spot ulcers.
1.5 = Haemorrhagic streaks.

2 = Ulcers ≥ 3 but ≤ 5.
3 = ulcers > 5.

The percentage ulcer protection $= \frac{uc - ut}{uc} \times 100$

Where Uc = ulcer index of treated group.
Ut = ulcer index of the control group.

B. Pylorus ligation model: Albino rats weighing between 150 – 200 g and each group containing 6 animals were divided into 6 groups.
Group A : Control group (vehicle)
Group B : Standard Lansoprazole (8mg/kg P.O.)
Group C : EEAC (200mg/kg P.O.)
Group D : EEAC (400mg/kg P.O.)
Group E : AEAC (200mg/kg P.O.)
Group F : AEAC (400mg/kg P.O.)

Experiment Procedure: Albino rats weighing between 150- 200 gm were divided into 6 groups of 6 rats in each. They are fasted in individual cages for 24 hr prior to the experiment with free access to water with measure to coprophagy. Group A served as normal control, which was given with vehicle only. Group B with standard drug , Group C, D, E & F treated with 200mg/kg and 400mg/kg dosages of EEAC & AEAC respectively. The various groups were treated with vehicle /extracts 30 min prior to pylorus ligation. Under light anesthesia, the abdomen was opened and the pylorus was ligated and sutured. 4 hour after ligation all the animals were sacrificed with excess of anesthetic ether and the stomach were dissected out. Gastric juice was collected into tubes and centrifused at 1000 rpm for 10min and volume was noted. The pH of the gastric juice is recorded by pH meter. The gastric content was subjected for analysis of free and total acidiy. The grandular portion of the stomach was opened along the greater curvature and ulcer index were determined. Mean ulcer score for each animal is expressed as ulcer index.

Reagent for biochemical estimation of free and total acidity of gastric juice

1) Freshly prepared 0.01N oxalic acid solution was used to standardize sodium hydroxide.
2) Freshly prepared 0.01N sodium hydroxide.
3) Topfer's reagent. It is dimethyl amino azobenzene 0.5% in absolute ethanol available in 100 ml package.
4) Freshly prepared 1% phenolpthelin solution prepared in 50% absolute ethanol.

Methods for biochemical estimation of free &total acidity: Gastric content collected from pylorus ligated rats was centrifuged and the volume of gastric juice was subjected to bio-chemical estimation as follows.

Determination of free and total acidity: 1 ml of gastric juice was pipette out out into a 100 ml of conical flusk, 2-3 drops of topfer's reagent aws added and titrated with 0.01N NaOH untill all traces of red colour disappear and the colour of the solution was yellowish orange. The volume corresponds to free acidity. Then 2-3 drops of phenolpthelin solution was added and titration was continued until a definite red tinge appears. Again the total volume of alkali added was noted. Now this volume is corresponds to total acidity.

Acidity was calculated by using this formula :

$$\text{Acidity} = \frac{\text{volofNaOH} \times \text{normalityofNaOH} \times 100}{0.1}$$

m.eq/lt/100g

Results

Anti-ulcer activity:

Ethanol Induced Anti-Ulcer In Rat's Model: The extracts of ripe fruits of *Ananas comosus* shows significant anti- ulcer activity in dose dependent manner, when compared to control which is evident by decrease in ulcer index. The ulcer index of ethanolic extracts of *Ananas comosus* at a dose of 200 mg/kg & 400 mg/kg.were found to be 2.87± 0.33 & 2.54± 0.21 respectively. The ulcer index of aqueous extracts of the *Ananas comosus* at dose of 100 mg/kg & 400mg/kg were found to be 2.57± 0.22 & 2.06± 0.35 respectively. Whereas standard (lansoprazole) mean ulcer index is 0.525 ± 0.0235. The aqueous extracts was found to be most potent. The result compiled in table no 1 and graphically presented in Fig- 1, 2.

Table No1: Effect of ethanolic and aqueous extracts of ripe fruits of Ananas comosus on ethanol induced ulcer in rats.

Sl.No.	Treatment	Dose	Ulcer index	% of ulcer protection
1	Control	D.W.	4.19±0.21	0%
2	Lansoprazole	8mg/kg	0.76±0.13***	83.65%
3	EEAC	200mg/kg	2.87± 0.33*	31.50%
4	EEAC	400mg/kg	2.54± 0.21**	39.37%
5	AEAC	200mg/kg	2.57± 0.22**	38.66%
6	AEAC	400mg/kg	2.06±0.35***	51.95%

Values are expressed as mean ± S.E.M., n=6, significant at ***P < 0.001, and *P<0.05 when compared to control group. Standard Drug ; Lansoprazole (8mg/kg).

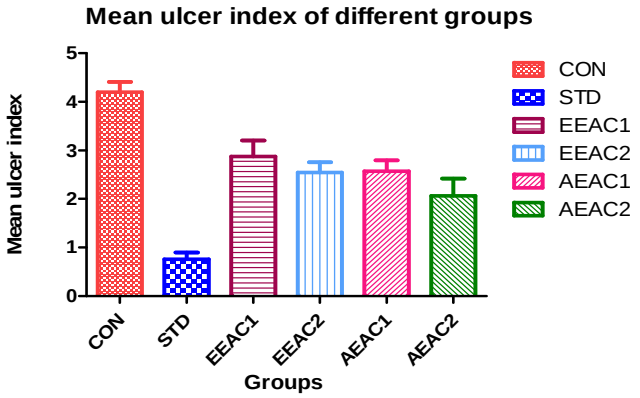

Fig 1 : Mean ulcer index of different groups in ethanol induced ulcer model

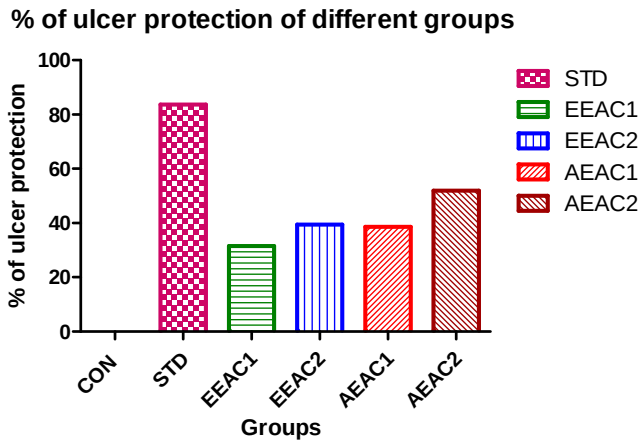

Fig 2:% of ulcer protection of different groups in ethanol induced ulcer model.

Pylorus ligated Anti-ulcer model: The extracts of ripe fruits of *Ananas comosus* shows significant anti- ulcer activity in dose dependent manner, when compared to control which is evident by decrease in ulcer index in pylorus ligation method. The ulcer index of ethanolic extracts of *Ananas comosus* at a dose of 200 mg/kg & 400 mg/kg, were found to be 3.245±0.288&2.598±0.116respectively. The ulcer index of aqueous extracts of the *Ananas comosus* at dose of 100 mg/kg & 400mg/kg were found to be 2.171±0.124&2.171±0.124respectively. Whereas standard (lansoprazole) mean ulcer index is 0.525 ± 0.0235. The aqueous extracts was found to be most potent. The result compiled in table no 2 and graphically presented in Fig- 3, 4,5,6,7 and 8.

Table No 2: Effect of ethanolic and aqueous extracts of *Ananas comosus* on pylorus ligated rats.

Groups	Dose	Gastric Content (ml)	pH	Free Acidity (meq/lt)	Total Acidity (meq/lt)	Mean Ulcer Index	% of Ulcer Protection
Control	D.W.	7.766 ±0.304	1.556 ±0.093	36.18 ±0.608	83.39 ±1.39	4.625 ±0.24	0
Standard	Lansoprazle 8mg/kg	4.183 ±0.308**	2.79 ±0.0862**	15.08 ±0.391***	36.97 ±0.90***	0.743 ±0.02***	83.93
EEAC1	200mg/kg	5.933 ±0.315***	1.99 ±0.053***	31.30 ±0.571*	67.32 ±1.71***	3.245 ±0.288**	29.83
EEAC2	400mg/kg	5.1 ±0.232***	2.12 ±0.086***	24.66 ±0.634***	57.48 ±0.97***	2.598 ±0.116***	43.83
AEAC1	200mg/kg	5.433 ±0.309***	2.11 ±0.073***	26.69 ±0.064***	59.66 ±1.29***	2.671 ±0.164***	42.24
AEAC2	400mg/kg	4.733 ±0.288***	2.38 ±0.03***	22.66 ±0.562**	51.14 ±1.56***	2.171 ±0.124***	53.05

Values are expressed as mean ± S.E.M., n=6, significant at***P<0.001, **P < 0.01 and *P <0.05 when compared to control group. Standard Drug; Lansoprazole (8mg/kg).

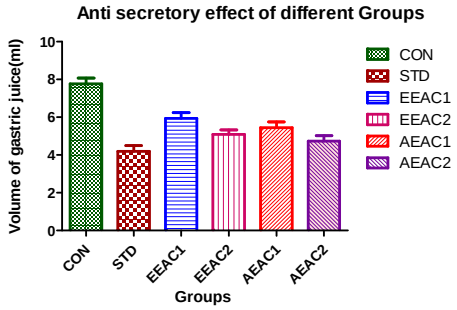

Fig 3 :Anti secretory effect in different groups in pylorus ligation model.

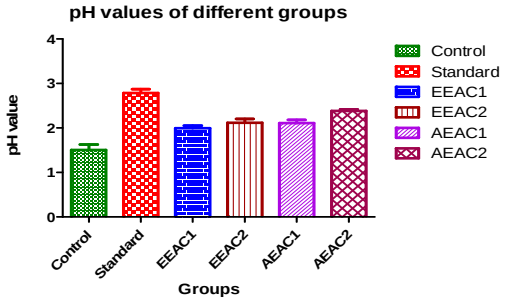

Fig 4: pH values of different groups in pylorus ligation model.

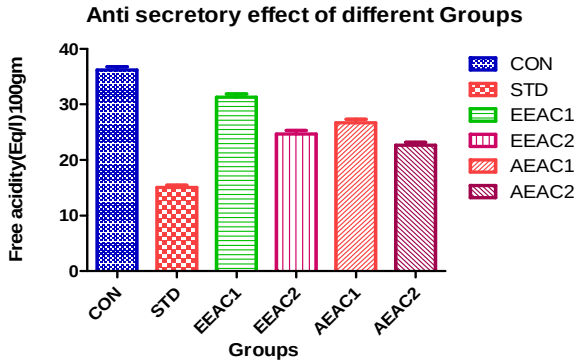

Fig 5: Avg. of free acidity of different groups in pylorus ligation model.

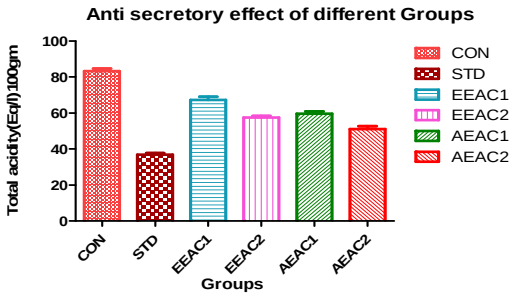

Fig 6: Avg of total acidity in different groups in pylorus ligation model

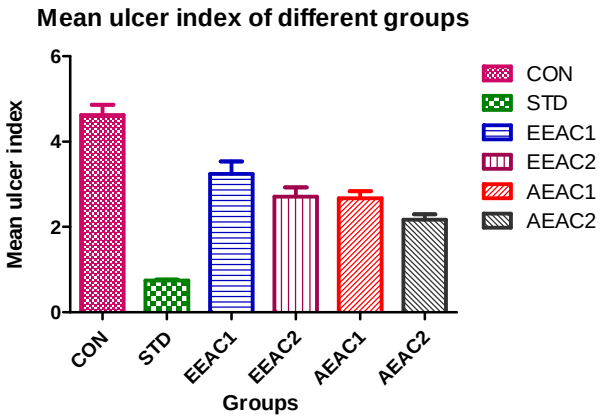

Fig 7: Mean ulcer index of different groups in pylorus ligation model.

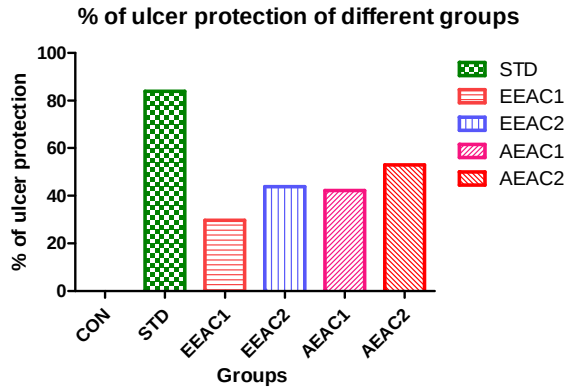

Fig 8: % of ulcer protection in different groups in pylorus ligation model.

Discussion: Peptic ulcer is one disease, which required treatment for chronic period. The usage of allopathic drugs for such a long time may results in adverse effect, adverse reaction, drug interactions etc[19-21]. Therefore several traditionally used drugs are being verified for this purpose and are available in the market for the purpose. In the present study one more herbal drug *Ananas comosus* which was used traditionally for various disease in all over world. In the present study the effect of *Ananas comosus* fruits was evaluated for its anti-ulcer,

The ethanolic and aqueous extract of *Ananas comosus* fruits was found safe up to 2000mg/kg in acute toxicity study.

Polyphenolic compounds were known to have anti-oxidant property and anti-oxidants are having gastroprotective role against various experimentally induced ulcer with a intention of verifying the claims of a native practitioner and correlate the results with the earlier reports.

The Polyphenolic compounds were known to have anti-oxidant property[9] and anti-oxidants are having gastro-protective role against various experimentally induced ulcer[18]. The phenolic extract of *Ananas comosus* reported for potent antioxidant properties [7, 8, 9].

In the present study an attempts is made to screen plant materials for the presence of various categories of gastroprotective property.

The plant contains β-anthocyanin, catechin and iso-catechin rather than from Vitamin C, E, Terpenoids, Flavonoids, Saponins, Tannins, alkaloids, anthaquinines, sterol, charbohydrates, oils and resigns [15].

The ethanolic and aqueous extracts were selected for further study.

The ethanolic and aqueous extracts of ripe fruits were subjected for screening anti-ulcer activity by using the following models.

1. Ethanol induced gastric ulceration.

Pylorus ligation induced gastric ulceration.

The parameters of the study were the reduction in the ulcer index in all the models and reduction in volume of secretion and acidity and increase in gastric pH in pylorus ligation model.

In ethanol induced gastric ulceration model, ethanol (1 ml/200 g), has induced severe ulcers as indicated by increase in red colouration, number of red spots, hemaorrhagic streaks and larger ulcers.

In the present study treatment with ethanolic and aqueous extracts of ripe fruits have shown significant and dose dependant gastric protective activity against ethanol induced ulcer as well as pyloric ligation method. Both extracts have been showed almost similar activities.

The gastroprotective activity against ethanol induced ulcers may be attributed to the anti-oxidant principle, probably the flavonoids and tannins[23].

In the pyloric ligation / shay rats preparation model, upon pyloric ligation there was significant increased in volume of gastric juice,

increased in free and total acidity, decreased in pH and there was an elevation in ulcer index[24-26]. Upon treatment with ethanolic and aqueous extracts of ripe fruit of ananas comosus fruits of the plants, has significantly reduced the ulcer index, volume of gastric juice, free acidity, total acidity and enhance the gastric pH in a dose dependent manner. Here both extracts were found to be almost similar activates.

In the pyloric ligation model the elevation in the gastric secretion and the ulcer may be due to the in balance between aggressive factors and mucosal integrity maintain by endogenous Defense mechanisms[27]. Several studies also indicated that prostaglandin may acts as gastroprotective as well as decrease in acid and pepsin secretion. This increased secretion of acid and pepsin may lead to auto-digestion of gastric mucosa and break down of mucosal barrier. In addition pylorus ligation may decrease GSH contain in gastric mucosa and increase mucosal lipidperoxidation[27].In the present study acid secretion was decrease and gastric pH was raised but there are reports that pepsin acts only at lower pH. Since there was an elevated pH, the pepsin becomes inactive and thereby there is a reduction in digestion of mucosal barrier. Since there is a report that the lipid peroxidation is increase due to pylorus ligation and lipid peroxidation is due to free radicals.

Overall our results were indicating that two extracts of the plants of the present study are possessing ulcer protective activity. It appears that the activity may be due to anti-oxidant property of plant and this anti-oxidant activity may be attributed to polyphenolic compound (flavonoids and tannins) of the plants.

Conclusion: The observation in this study confirms that *Ananas comosus*ripe fruits have gasrtopotective property that justified that the traditional claim. The gastro-protective activities may be attributed to the presence of flavonoids and tannins mediated by antioxidant property.

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