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EVALUATION OF ANTIULCER ACTIVITY OF ASPARAGUS RACEMOSUS ON EXPERIMENTAL ANIMALS

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ABSTRACT

In present, peptic ulcer is a worldwide problem and its prevalence is quite high in India. Some present data shows that in different part of India its occurrence is 4 to 10% per thousand populations. The exact etiology of peptic ulcer is not known, the disease results in chronic suffering, loss of working hours and occasional fatality. The life time prevalence of peptic ulcer disease (PUD) in United States is approximately 12% in men and 10% in women. In USA, approximately 4 million individuals have peptic ulcers, while each year about 350,000 new cases are diagnosed; about 100,000 patients are hospitalized and at least about 3000 people die as a result of the disease. Smoking habit, alcoholism, stress & spicy food make the severity of the disease which leads serious complication of ulcer. Asparagus Racemosus is one of the most commonly use in different medical conditions has been documented. Traditionally, Asparagus Racemosus are beneficial in the treatment of diseases related to lungs & airways, blood, skin, stomach, intestines, and liver. As no reports are available on the possible antiulcer effects of Asparagus Racemosus. The present work was carried out to antiulcer activity of Asparagus Racemosus by ethanol induced gastric ulcers in wister albino Test drug Asparagus Racemosus (low dose) and Asparagus Racemosus (high dose) have

shown result in comparison to standard drug omeparazole and control drug. Thus study has provided documentary evidence for antiulcer property of Asparagus Racemosus for its activity.

KEYWORDS: Asparagus Racemosus, Shatavari, Peptic Ulcer, Ulcer Score, Asparagaceae, Omeprazole.

1. INTRODUCTION

Peptic Ulcer

Ulcer is defined as 'general destruction of stomach or duodenum or both; bathed by gastric juice which is acidic in nature. Ulcer in the mucosal layer of stomach tends to be of recurring nature in presence of stress**.** Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance.

A peptic ulcer, also known as PUD or peptic ulcer disease, is the most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. It is defined as mucosal erosions equal to or greater than 0.5cm. Gastric ulcer is a breach in continuity of lining epithelium in those parts of the digestive tract, which are continuously exposed to gastric juice containing acid and pepsin. The term peptic ulcer refers to those ulcers which occur in either the stomach or first part of small intestine that leads out of stomach called duodenum. Peptic ulcers are deep gastrointestinal erosion disorder that involves the entire mucosal thickness, penetrating the muscular mucosa. It is generally accepted that it results from an imbalance between aggressive and defensive factors.

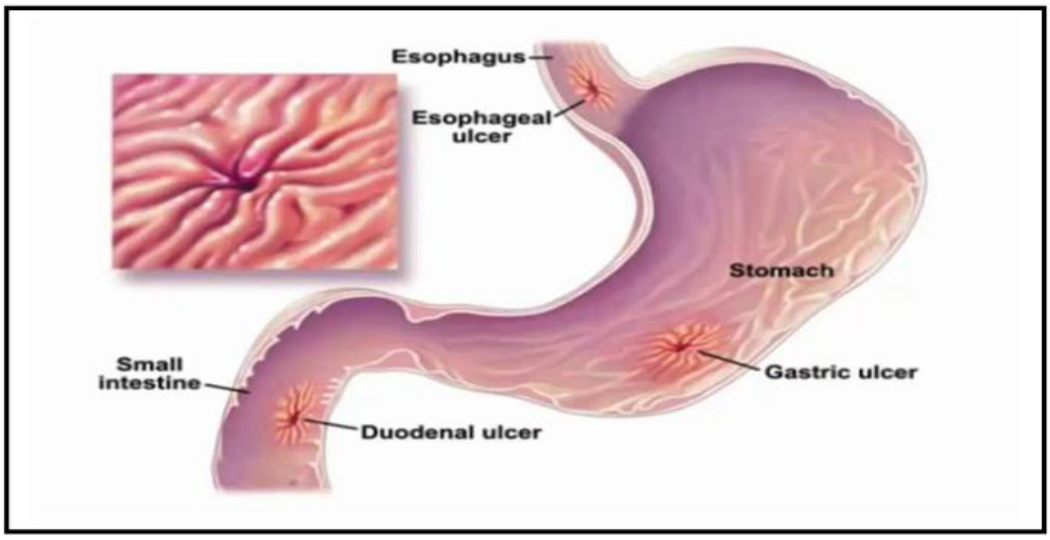

Figure 01: Peptic ulcer Disease.

Classification of Peptic Ulcer

I) By Region/Location

- Duodenum (called duodenal ulcer)
- Oesophagus (called esophageal ulcer)
- Stomach (called gastric ulcer)

II) Modified Johnson Classification of peptic ulcers

Table 1: Modified Johnson Classification of peptic ulcers.

Type I	Ulcer along the body of the stomach. most often along the lesser curve at incisura angularis along the locus minoris resistentiae.
Type II	Ulcer in the body in combination with duodenal ulcers. Associated with acid over secretion.
Type III	In the pyloric channel within 3 cm of pylorus. Associated with acid over secretion.
Type IV	Proximal gastroesophageal ulcer.
Type V	Can occur throughout the stomach. Associated with chronic NSAID use (such as aspirin).

Causes

- High acid and peptic content
- Irritation
- Poor blood supply
- Poor secretion of mucus
- Infection, *H. pylori*
- Other diseases and factors
- Cigarette smoking
- Psychological stress
- Alcohol
- NSAIDs
- Diet
- Severe physiologic stress
- Burns
- CNS trauma
- Surgery
- Severe medical illness.

Pathophysiology of Peptic Ulcer

The pathophysiology of the gastric ulcer has not been completely elucidated, but it is known that an imbalance between aggressive factors (acid and pepsin secretion) and cytoprotective factors of the gastric mucous membrane (mucus and bicarbonate secretion) result in gastric ulceration. It is also known that several endogenous factors are involved in the pathophysiology of the gastro-protection which includes prostaglandin E_2 (PGE_2), somatostatin, nitric oxide (NO) and sulthydryl compounds. The etiology of gastric ulcer involves environmental factors such as alcoholic beverages and non-steroidal anti inflammatory drugs (NSAIDs) use, Helicobacter pylori, genetic factors, among others Alcohol is known as a necrotizing substance and its excessive ingestion may result in gastritis, characterized by mucous membrane edema, sub epithelial haemorrhages, cell exfoliation and inflammatory cells infiltration. Thus, gastrointestinal tract diseases related to excessive alcohol use play an important role in the clinical gastroenterology.

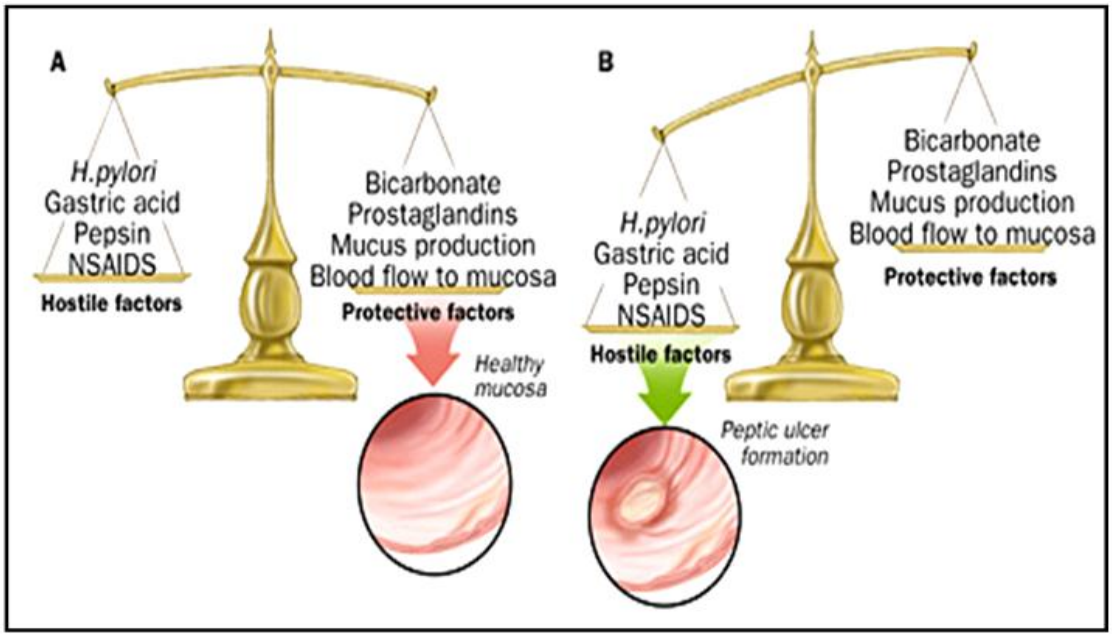

Fig. 02: Protective factors *vs.* hostile factors.

1. Association with Gastric acid secretion

Most peptic ulcers occur in the presence of acid and pepsin when HP, NSAIDs, or other factors distrupt mucosal defence and healing mechanisms. A minimal level of gastric acid secretion is necessary for the formation of peptic ulcer. Thus gastric acid serves as a cofactor with H. Pylori infection and NSAID use. Factors responsible for acid hyper secretion include increased parietal cell mass, increased basal secretory drive, and increased postprandial

secretory drive. Acid hyper secretion may also be consequence of HP infection. Hyper secretion of acid is the primary pathogenic mechanism in hypersecretory states such Zollinger-Ellison syndrome.

2. Association with *H. Pylori*

The strong association exits between H, pylori (HP) and PUD. Virtually all patients with GU who are not taking NSAlDs have evidence of HP infection and antral gastritis. A similar relationship between HP and PUD is difficult to confirm, in part because only a small number of individuals infected with HP actually develop an ulcer.

HP is an acid-labile, spiral-shaped gram negative rod that resides between the stomach or any location where gastric epithelial is found. The shape and motility of the bacterium permit penetration of the mucus layer where the local pH is less acid. Before HP enters the mucus, it produces large amounts of urease, which breaks down urea in gastric juice and converts it to ammonia and carbon dioxide. This metabolic process continues after HP reaches the "safe haven" of the mucus. Transmission of the organism in Western countries is thought to be person to person via the faecal-oral and oral-oral routes as humans are the only reservoir of infection. Most infections are passed from parents to their children. It is also possible to transmit HP via infected instrument such as endoscopes. It appears that HP infection rates correlate with lower socioeconomic status and health standards.

2. Plant Profile of Asparagus Racemosus

Introduction to Plant

Botanical name: *Asparagus racemosus (willd)*

Family: *Liliaceae*

Figure 03: Asparagus racemosus.

Synonym: Asparagus rigidulus Nakai, Protasparagus racemosus (willd).

Part Used: Roots, Leaves, flowers**,** Fruits.

Habitat

This climber growing in low jungles is found all over india; especially in Northern india. The plant is a climber growing to 1-2 in length found all over india.

Asparagus Racemosus

Asparagus racemosus (satavar, shatavari, or shatamull) is a species of asparagus common throughout Nepal, SriLanka, India and the Himalayas. It grows 1-2 m (3 ft 3 in 6 ft 7 in) tall and prefers to take root in gravelly, rocky soils high up in piedmont plaints, at 1,300-1,400 m (4,300-4,600 ft) elevation. It was botanically described in 1799. Because of its multiple uses, the rise. Because of destructive harvesting, combined with habitat destruction, and deforestation, the plant in now considered "endangered" in its natural habitat. Shatawari has different names in the different Indian languages, such as shatuli, vrishya and other terms. In Nepal it is called kurilo. The name "shatawari" means "curer of a hundred diseases".

Genral information

Asparagus racemosus is the ayurvedic name of an herb known as satawar, satavari in Hindi. *Asparagus racemosus* is an indigenous medicinal plant of the family Liliaceae is important for its sapogenin content, the precursor of many pharmacologically active steroids This species occurs widely throughout the tropical and subtropical region. Several authors have shown that the species from different localities often differ in their chemical constituents and contents.

The Latin name of the plant is *Asparagus racemosus* and it belongs to Liliaceae family.

Scientific classification

Kingdom –Planate. Division– Angiosperms Class – Monocots. Order – Asparagales Family – Asparagaceae Genus – Asparagus Species – Asparagus racemosus.

Vernacular names

The various vernacular names of plant are as follows:

- English – Willd asparagus
- Hindi – Satavar

- Bengal – Shatamuli
- Gujrati- Ekalkanto, Satavari
- Kannad– Callagandda
- Tamil – satavali
- Marathi name – Satawarmul, Satavari
- Sanskrit- Shatmili, Satavari
- Telugu – Satavari, Callagad
- M.P.-Narbodh, Satmooli
- Rajasthan – Satawar
- Oriya – Chhotaru, Mohajolo

Phytochemical constituents

Asparagus racemosus possesses some phytochemical constituents which have medicinal values. The composites of *Asparagus racemosus* such as alkaloids, saponins, tannins, flavonoids, phenolic compounds were isolated and characterized with the help of chromatographic separation techniques and their structures were explained by the using nuclear magnetic resonance techniques.

A lot of chemical analysis has been carried out on the roots of Asparagus racemosus. The mojor reported constituents included saponins, shatavarinI, shatavarin II, shatavarin, shatavarin IV alkaloids, proteins, starch and tannins. Isoflavones including 8-methoxy-5,6,4'-trihydroxyisoflavone 7-o-beta D-glucopyranoside and Asparagamine, a polycyclicalkaloides were also isolated.

The leaves contains steroids diosgenin along with rutin; a flavanoid- glucoside- quercetin-2-glucoronide, ferulic, caffeic and chlorogenicacides. The flowers containsquercetin, hyperoside and rutin; fruit contains glycoside of quercetin, rutin, hyperoside, fuly ripe fruits contain cyaniidine-3- glucorhanoside. The shoots of the plant contain a bitter principle, 22-spirostan 3β-o1, sarspogenin, rhamnose, xylose and glucose.

Kaepfrol is another component along with that of sarsapogenium which can be isolated from the woody portions of the tuberous roots.

Some of the essential fatty acids like gamma linoleinic acids, disogenin are also obtained from this plant.

Morphological characters

Shatavari is a highly branched, consisting of thorn under shrubs. It is a woody climber plant which is 1-2 m in distance and readily grows up a documentation or over other plant. The leaves of the shatavari looks like pine needles, uniform and small in size. The roots of the plant have a finger like structure and are clustered in nature. It has a tiny white coloured flower while the plant itself is bittersweet in taste.

Botanical description

Asparagus racemosus is plant with a woody stem that sends runners out, has needle like leaves with small white flowers, (Aviva Romm, 2010), Scandant, much branched spinous under shrub with tuberous, short root, stock bearing numerous fusiform uberous roots 30-100cm thick leaves reduced to minute chaffy scales & spines. Cladodes acicular 2-6 hate, falcate finely acuminate flower white, berries 7mm india meter, globose, 1-seeded, red.

Pharmacological Action

1. Gastrointestinal effects

The powdered dried roots of asparagus racemosus promote gastric emptying in healthy volunteers and its action comparable with that of the synthetic dopamine antagonist metoclopramide. It has been report that A. racemosus along with Terminalia *chebula* protect gastric mucosa against pentagastrin and carbachol induced ulcers, by significantly reducing both severity of ulceration and ulcer index.

2. Galactagogue effect

Alcoholic extract of *Asparagus racemosus* have a significant effect on lactating mother to increase milk production and have been observed along with increase growth of the mammary gland alveolar tissue and acini. The growth of lobuloavelar tissue and milk secretion in the estrogenic primed rats was thought to be due to the action of released corticoids or prolactic. The galactagogue effect has also been studies in buffalo. the effect was evaluated in 60 lactating mothers by measuring the change in the prolactin hormone level. The study shows that the oral administration of *A.racemosus* led to thrice increase the level of prolactin than of the control group.

3. Immunomodulatory activity

The use of asparagus racemosus dried root powder modulates the action of the immune system. That in turn, decreases the inflammatory response. It induces the immune system to

fight against immune deficiencies (like AIDS), infections and cancer. It may be helpful in obtaining higher protective antibody against different vaccinations including more effective cell mediated immune response for protective against various bacterial, viral, and other diseases. Several workers has studies the effect of Asparagus racemosus root extract in augumentation of humoral and cell mediated immune response providing better protection level against infections.

4. Anticancer activity

Natural products have long been used for treatment against cancer. There are at least 10000 species of plants. Documented to have anti- cancerous properties. the isolated shatavarin IV with AR-2B containing 5.05% shatavarin IV showed potent cytotoxicity. It showed increase in non viable cell count when study. Hence from various in vitro and in vivo models it can be concluded that the root extract the plant which contain shatavari IV fraction exhibits significance activity against cancer cells.

5. Cardiovascular effects

Increase in serum lipid levels especially cholesterol along with the generation of reaction oxygen species are the major reasons for development f coronary artery disease and atherosclerosis “Abana” a herbo- mineral formulation containing 10 mg *Asparagus racemosus* extract per tablet, as found to have significant hypocholeroacemic effect in rats and therefore established a potential for use as a cardio- protective agent.

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7. Antioxidant action

Antioxidants are the moieties which are involved in the prevention of cell damage, common pathway for many diseases. As given by Aarati K the Methanolic extract of the root possess

significant anti-oxidant properties when administered through the oral method. The levels of enzymes like superoxidase dismutase, catalase and ascorbic acid increase with significant reduction in the lipid peroxidation. The antioxidant properties were mainly exhibited due to the presence of Isoflavons.

8. Antiulcer effect

Asparagus racemosus is an effective antiulcerogenetic agent whose activity can easily be compared with that of ranitidine hydrochloride *Asparagus racemosus* causes an inhibitory effect on release of gastric hydrochloric acid, and protects gastric mucosal damage. Hence the roots of the Shatavari plant in the form of powder can be administered to chronic ulcer patients along with other patients.

9. Antidiarrhoeal effect

Diarrhoea can be classified as one of the major problems faced mainly in the developing countries with an estimated death rate of 2.2 million people globally, mainly in the developing countries. The fatal cases can be mainly seen in the children with the 5 years of age. The ethanol and aqueous extracts of roots of *A. Racemosus* exhibited a significant anti-diarrhoeal activity against the castor oil induced diarrhoea in the rats. Studies have shown that the action of prostaglandin E caused the diarrhoea in the test subjects, hence it can be said that the action of this can be to prevent the biosynthesis of prostaglandin which in turn inhibits the diarrhoeal effect.

10. Antitussive effect

A. racemosus has been commonly used in the treatment of cough and in minor upper respiratory tract infection, exhibiting the anti-tussive properties. In the experimental setup by Akansha Singh and Sinha (2014) the methanol extract of the roots showed activity against sulphur induced cough in mice which was likened to the codeine phosphate, a drug obtained from opium.

Hence this extract can be used against the opium based drugs, since there are no side effects like nausea, sweating, tiredness which can be observed by use of codeine phosphate associated drugs.

11. Aphrodisiac activity

Lyophilized aqueous extracts roots of *Asparagus racemosus* have sexual behavioural effect in male albino rats. Administration of the aqueous extracts has pronounced anabolic effect in treated animals as evidenced by weight gains in body and reproductive organs. There was a significant variation in the sexual behaviour of animals as reflected by reduction of mount latency ejaculation latency, postejaculatory latency, intromission latency.

12. Anti- dyspepsia effects

Dyspepsia is the condition characterised by the inability to digest, commonly stated as impaired digestion which is frequently associated with the gastritis. In small minority it can as well be the first symptom for gastric ulcer and cancer. The Shatavari was compared with that of Metoclopramide, which is a synthetic dopamine used in patients with dyspepsia to increase the rate of emptying of the abdomen. Hence it was found that the rate of emptying did not differ significantly. It can be reasoned that the extract can be utilised for the treatment and the side effects from the use of metoclopramide like drowsiness can be averted.

13. Problems association with menstruation

The constituents of *Asparagus racemosus*make it useful in menstrual disorders such as dysmenorrhea, premenstrual syndrome, irregular bleeding during peri-menopausal period and also in situations after menopause. *Asparagus racemosus* contain saponins which hinder the oxytocic activity on uterine musculature, thereby maintain the spontaneous uterine motility, confirming its utility in dysmenorrhea which comprises of painful menstruation without significant pelvic pathology.

14. Problems associated with menopause

A common practice to relieve menopausal symptoms is to administer hormone replacement therapy, which is not free from adverse effect. Thus women are turning to natural medication in an effort to deliver adependable alternative to synthetic steroidal hormones. *Asparagus racemosus* being known sources of phytoestrogen can be effective in reducing adverse menopausal symptoms (The chemical entities from plants which mimic hormone are called phytoestrogens). These are weaker than natural estrogen in action.

3. MATERIAL AND METHOD

Material

Collection & Authentication of Plant Material

The medicinal Leaves of *Asparagus racemosus* have been collected from Government college of pharmacy, Amravati, India 444 601, jan 2018. The plant was authentified by Vidybharti mahavidhyalaya, Amravati, 444 602. With their truthful label.

Drugs and Chemicals

A. Standard Drug

Omez 20 Tablet (Omeprazole 20mg). This is an allopathic formulation of Dr Reddy's Lab, Baddi-solan, Himachal Pradesh. Used mainly intreatment of pepticulcerand acidity.

B. Other Chemicals

Ethanol (90%), Anaesthetic ether, HCl, 0.1N NaOH, Phenolphthalein indicator, Formal saline all these chemicals were procured from S.D. Fine Chemical Ltd. Mumbai, Maharashtra. The chemicals used and other solutions were all of analytical grade. All drugs and reagents were prepared immediately before use.

Methods

Extraction method

For the extraction of plant material the soxhlet extraction method may be used. Requirements of extraction process are as follows:

A] Solvent: Ethanol was used as solvent.

B] Apparatus: Soxhlet Extraction Assembly.

Procedure

- Asparagus racemosus leaves are properly washed with tap water and rinsed with distilled water.
- The rinsed leaves were shade dried and powederd.
- The powdered material was subjected to solvent extraction with ethanol in soxhlet apparatus at room temperature.
- The filtrate was concentrated by using rotary vacuum evaporator.
- The extract obtained with each solvent was weighed and the percentage was calculated in term of dried weight of the plant material.
- The color and consistency of the extract were also noted.

The extract was stored in refrigerator and reconstituted in gum acacia before administration to animals.

Experimental Animals

The experiment is performed on albino Wistar rats (weighing 150-200 g), which are obtained from the animal house of Department of Pharmacology, Vidyabharati college of pharmacy, Amravati. All the animals are acclimatized to the animal house prior to use. They are kept in cages in animal house with a 12 h light: 12 h dark cycle. Animals are fed on pellets and tap water ad libitum. The care and handling of rat were in accordance with the internationally accepted standard guidelines for use of animals (CPCSEA). Permission and approval for animal studies was obtained from the Institutional Animal Ethics Committee (IAEC) of Vidyabharti college of Pharmacy, Amravati. SGB Amravati University. (1504/Po/Re/S/11/CPCSEA-09/08/2016).

A. Selection of Animal species & housing

For the acute toxicity study the Female rats were used, as Female rats are more sensitive than Male rats. All the test animals were kept in separate cages at least 5 days before the commencement of toxicity test. Animals were maintained at 22 ± 30^0C in (12:12) light & Dark cycle with free access of Food and Water.

B. Test procedure

The required dose is administered in animal one at a time by using oral gavage The animal (Rats) were fasted overnight but water was not withdrawn. The fasted body weight of rat is determined and Dose is calculated on body weight basis after administration of *Asparagus racemosus* extract the food is withheld for further 3-4 h. For limit test 2000 mg/kg dose was administered in one animal and then the animal was observed for mortality for a period of 48 k the tested rat was servived therefore test was continue by taking 4 more animals.

In main test dose of 250,500, 1000,1500 was selected and was administered in animal one at a time. The animal was observed for any toxic symptoms initially for 1h. interval for 4 h. then periodically for up-to 14 days.

C. Selection of Dose groups

1. On the basis of acute toxicity study data. It was conclude that LD50 of *Asparagus racemosus* ethanolic extract is more than 2000mg/kg.

2. 200 mg/kg of dose was selected and was examine for its Antiulcer efficacy. it was found to be effective.
3. Therefore the test groups were divided as 200mg (low dose), 400 mg (medium dose), 800 mg(high dose).

Determination of Antiulcer Activity

The Antiulcer potential of ethanolic roots extract of *Asparagus racemosus* has been carried out by using Ethanol induce ulcer model in Albino wistar rat of either sex weighing 150-250 gm.

Ethanol Induced Ulcer

Rats were divided into five groups with six animals in each. The rats were fasted 24 h prior to start of experiment. Then the first groups receive an oral dose of the vehicle (10 ml/kg), in the second group Omeprazole (20mg/kg) & 3th, 4th and 5th group receive 200, 400 and 800 mg/kg body weight of root extract of *Asparagus racemosus.*

After 60 min, all groups were orally treated with 1 ml of ethanol solution for gastric-ulcer induction. Animals were killed 1h after the administration of ethanol and the stomachs were excised and gastric damage (score for ulcer) determined as described below.

Treatment Protocol

Briefly, the animals were divided into five groups (n = 6) and treated with the respective test solutions as given below:

1. Group 1 (negative control group) –Ethanol + vehicle.
2. Group 2 (Standard group) - Ethanol + 20 mg/kg Omeprazole.
3. Group 3(Low dose group) - Ethanol + 200 mg/kg ELAR.
4. Group 4 (Moderate dose group) - Ethanol + 400 mg/kg ELAR.
5. Group 5 (High dose group) - Ethanol + 800 mg/kg ELAR.

Ulcer Scoring

Scoring for the ulcer is made according to the severity of ulcer as follows:

Table 2: Observation Table for Scoring of Ulcers Score.

Score	observation
0	Normal, No ulcer
0.5	Red Coloration
1	Spot ulcer
1.5	Hemorrhagic Streak
2	Deep ulcer
3	Perforation

Determination of Ulcer Index

After scoring Ulcer according to their severity, the mean ulcer score for each animal was expressed as ulcer index. Ulcer index was measured by using following formula:

Ulcer Index (UI) = UN + US + UP X 10^{-1}

Where,

UI = Ulcer Index,

UN = Average number of ulcers per animal,

US = Average number of severity score,

UP = Percentage of animals with ulcers.

% Inhibition of Ulceration

Percentage inhibition of ulceration was calculated as below:

% Inhibition of Ulceration = (Ulcer index Control-Ulcer index Test) ×100/Ulcer index Control.

Determination of Total Acidity and pH

The stomachs were removed and the content was subjected to centrifugation at 3000 rpm for 10 min. The total acidity of the gastric secretion was determined by titration with 0.01 N NaOH and phenolphthalein as indicator.

The total acidity is expressed as equiv./1using the following formula:

$$\text{Total Acidity} = \frac{n \text{ X } 0.01 \text{ X } 40 \text{ X} 1000}{0.1}$$

Where,

n is volume of NaOH quantified,

40 s the molecular weight of NaOH,

0.01 is normality of NaOH and

1000 is the factor represented in litre.

Determination of pH

pH of the gastric secretion was recorded with calibrated pH meter.

Statistical Analysis

The data were expressed as mean±SEM. Results were analyzed statistically by One-way ANOVA followed by DUNNETT's TEST using Primer of Biostatistics, Version 4, The difference was considered significant if $P<0.05$.

4. RESULTS

- **Extraction Yield**
- Wt of dried leaves powder :- 100gm
- Wt of extract obtained :-10.66gm

The yield of Leaves Extract of *Asparagus racemosus* was 10.66 gm for 100gm leaves powder, i.e 10.66%.

Therefore,

$$\textbf{\% practical yield} = \frac{\text{Practical yield}}{\text{Theorotical yield}} \times 100$$

$$= \frac{10.66}{12.5} \times 100$$

$$= \textbf{85.28 \%}$$

The % practical yield of Ethanolic leaves extract of *Asparagus racemosus* found to be 85.28%.

Phytochemical Investigation

Table 03: Observation table for Phytochemical Investigation of *Asparagus racemosus.*

Sr. No.	Natural Product	Test Performed	Inference
1	Alkaloid	Dragendorff's Test	-
		Mayer's Test	-
		Hager's Test	-
2	Steroids	Salkowask'y test	+
3	Phenol	Lead acetate test	+
4	Flavone	Shinoda test	+
5	Enzyme test	Catalyst test	+
6	Carbohydrate	Molisch's test	+
		Fehling's test	+
		Benedict's test	-
7	Saponine	Soap Formation with water	+
8	Glycoside	Brontragar's test	-
		Flavonoid Glycoside	-
9	Tannin	Gllotannin	+
		Catechin	+
10	Protein and Amino Acid	Ninhydrin test	+
11	Quinones		-
12	Terpenoids		-

+ Indicates presence

- Indicates absence

Phytochemical testing was carried to find out the secondary metabolites because secondary metabolites possess biological activity. The data of above table reveals that steroids, flavonoids, carbohydrates, saponin, tannins & phenolic compounds were present in *Asparagus racemosus leaves.*

Acute Oral Toxicity Study

In order to decide the dose range of herbal formulation, toxicity study was carried out as per OECD guidelines No 425 (Annexure 2c, Annexure 2d, 2001) of CPCSEA for acute oral toxicity. No mortality and no sign of toxicity were found after the administration of 250 mg/kg; 500mg/kg; 1000mg/kg; 1500 mg/kg and 2000 mg/kg of herbal formulation by oral route. No death observe up to dose of 2000 mg/kg. Therefore the LD_{50} of ethanolic leaves extract of *Asparagus racemosus* found to beyond 2000 mg/kg.

So, for present experimental studies the 200 mg/kg as a low dose, 400 mg/kg as a medium dose & 800 mg/kg as a high dose of *Asparagus racemosus* was selected.

Pharmacological Study

Ethanol Induced Ulcer

A. Ulcer score

Table 04: Mean Ulcer Score in Ethanol induced ulcer.

Treatment Group	Dose mg/kg	Mean Ulcer Score
Control Group	10ml/kg	2.4166±0.2713
Omeprazole	20 mg/kg	0.1666±0.1667
ELAR	200 mg/kg	1.5833±0.2007
ELAR	400 mg/kg	1.666±0.2108
ELAR	800 mg/kg	0.7500±0.1708

Each group consist of six animals, Data is presented in mean±SEM.

Here ** means significant difference ($p<0.05$) as compared to Negative control group.

Ns= no significant.

ELAR= Ethanolic leaves Extract of *Asparagus racemosus.*

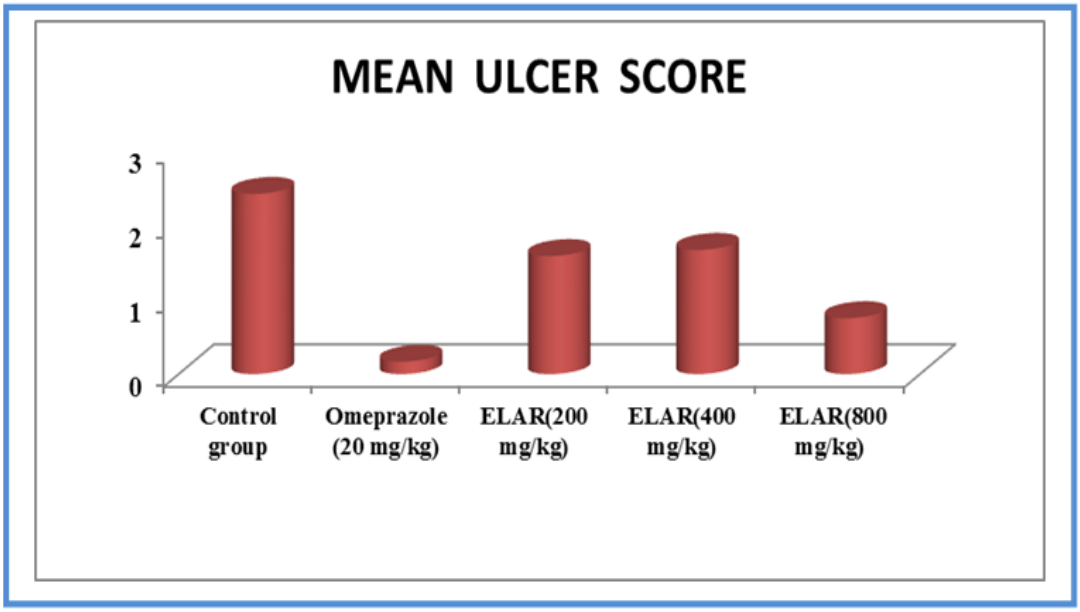


Figure 04: Bar chart of ulcer score in Ethanol induced ulcer.

Ulcer score is the counting of spots and severity of damage in gastric part by any moiety such as ethanol. This present study showed that all drug groups showed protection against ethanol damage by significant level ($P<0.05$) as compared to vehicle treated group. Single drug treatment (200 mg/kg, 400 mg/kg and 800 mg/kg of ELAR) was effective up to significant level ($P<0.05$) as compared to vehicle treated group.

B. Ulcer number

Table 05: Observation table for Ulcer number in Ethanol induced ulcer.

Animal	Control group	Std.(omeprazole 20 mg/kg)	ELAR (200 mg/kg)	ELAR (400mg/kg)	ELAR (800 mg/kg)
1	7	0	1	1	0
2	5	0	2	1	1
3	7	0	2	2	1
4	4	1	2	2	0
5	6	0	1	1	1
6	7	1	2	0	1
mean±SEM	6.000±0.5165	0.3333±0.2108	1.667±0.2108	1.167±0.3073	0.6667±0.2108

Ulcer Index and % Inhibition of Ulcer

Table 06: Observation table for Ulcer Index and % of Inhibition in Ethanol induced ulcer.

Group	Treatment	Ulcer Number	Mean ulcer score	Incidence of ulcers (%)	Ulcer Index	% Of Inhibition
Control	Vehicle 10 ml/kg	6.000±0.5165	2.4166±0.2713	100 %	11.20	-
Standard	Omeprazole 20 mg/kg	0.3333±0.2108	0.1666±0.1667	33.33 %	3.56	68.25
ELAR	200 mg/kg	1.667±0.2108	1.5833±0.2007	100 %	10.7	4.46
ELAR	400 mg/kg	1.167±0.3073	1.666±0.2108	83.33 %	9.04	19.28
ELAR	800 mg/kg	0.6667±0.2108	0.7500±0.1708	66.66 %	7.13	36.33

Each group consist of six animals, Data is presented in mean±SEM. Here ** means significant difference ($p<0.05$) as compared to Negative control group. Ns= no significant. ELAR= Ethanolic leaves Extract of *Asparagus racemosus.*

A. Ulcer Index

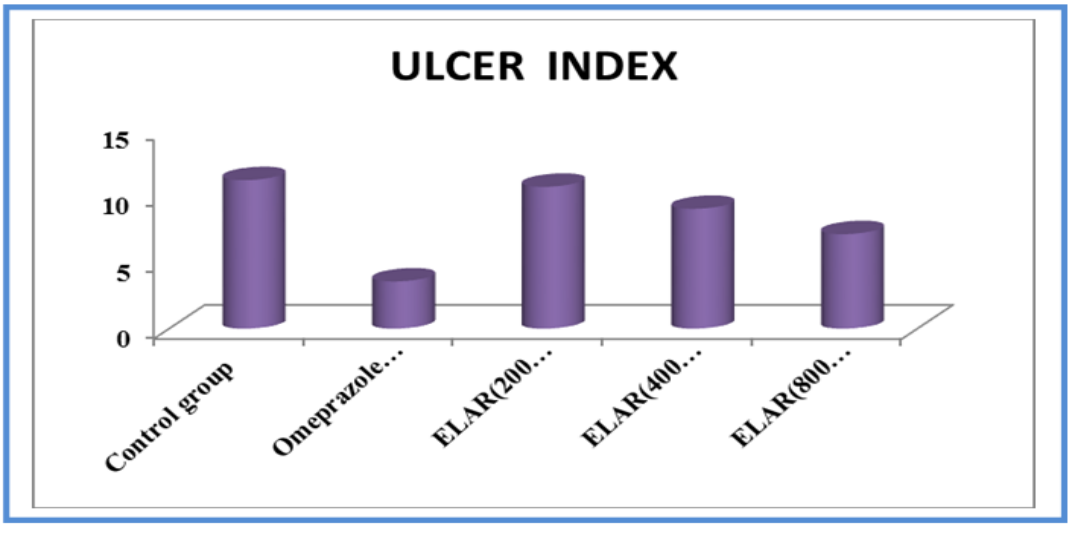


Figure 05: Bar chart of ulcer Index in Ethanol induced ulcer.

B. % Inhibition of Ulcer

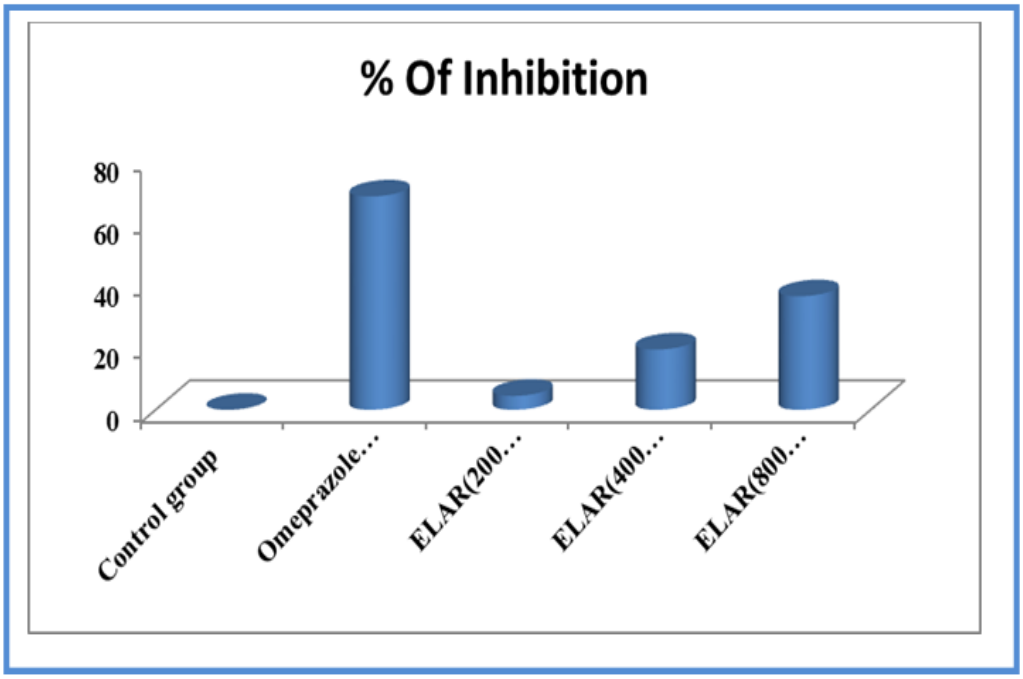


Figure 06: Bar chart of % Inhibition of Ulcer in Ethanol induced ulcer.

The % of occurrence of ulcers in stomach is significantly inhibited by the standard drug (omeprazole) and ELAR in dose dependent manner.

Determination of PH and Total Acidity of Gastric Juice

Table 07: Observation of pH, Free Acidity, Total Acidity and Volume of Gastric Juice.

Treatment group	Ph	Free acidity Meq/L	Total acidity Meq/L	Volume of Gastric Juices (ml)
Control group	1.36± 0.049**	33.33±0.66	84.83±1.19	6.77±.09
Omeprazole (20 mg/kg)	2.85±0.089**	19.00±0.68***	45.67±.91***	4.38±.10***
ELAR (200 mg/kg)	2.36±0.045**	26.83±0.60***	61.67±.95***	6.20±.05***
ELAR (400 mg/kg)	2.66±0.072**	24.50±.88***	49.00±1.15***	5.15±.04***
ELAR (800 mg/kg)	2.75±0.068**	22.67±.71***	48.67±.61***	4.88±.06***

Each Group consist of six animals, Data is presented in mean±SEM. Significant at P<0.05*, 0.01**, ns= not significan.

ELAR= Ethanolic Leaves Extract *Asparagus racemosus.*

A. Determination of pH of Gastric Juice

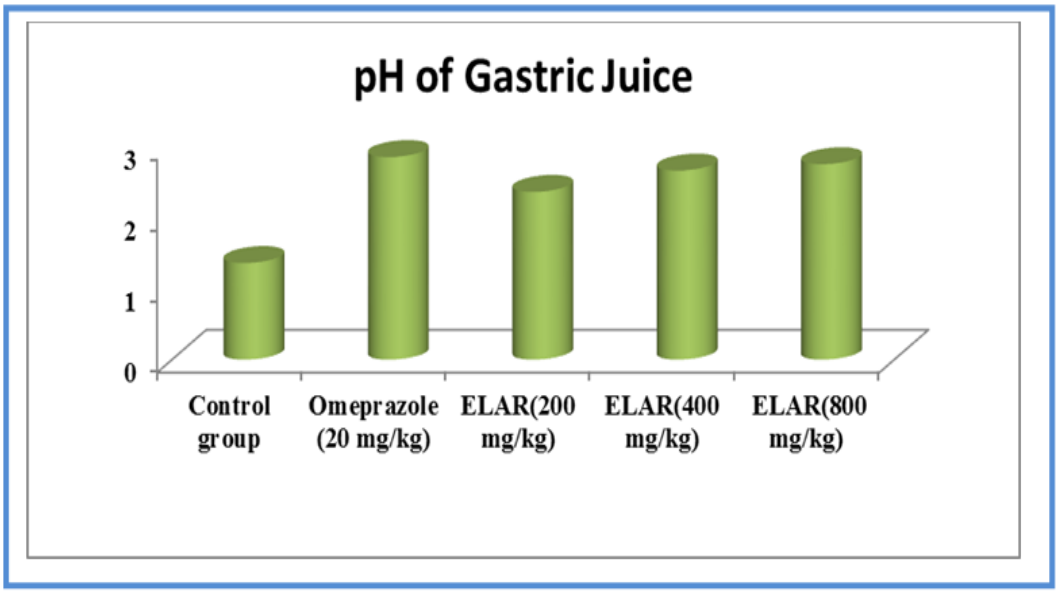


Figure 07: Bar chart pH of Gastric Juice.

Low pH is responsible for more damage in gastric portion. In this study the pH is increase by significant level in both standard (omeprazole) and test (200, 400 & 800 mg/kg of ELAR) group as compare to control group.

B. Free Acidity

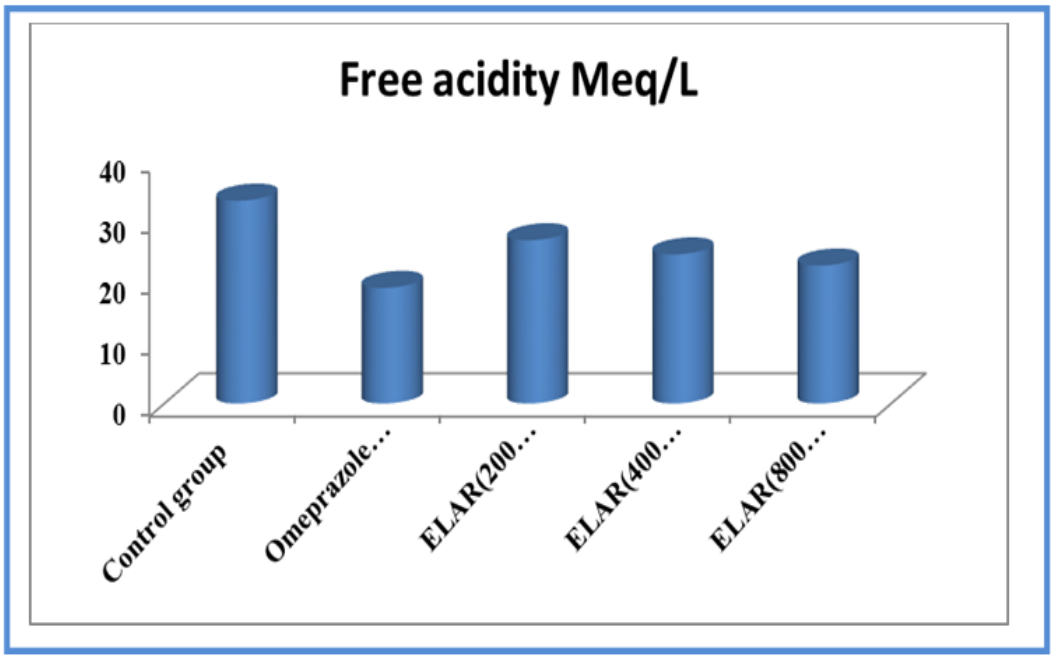


Figure 08: Bar chart of Free Acidity of Gastric Juice.

C. Total Acidity

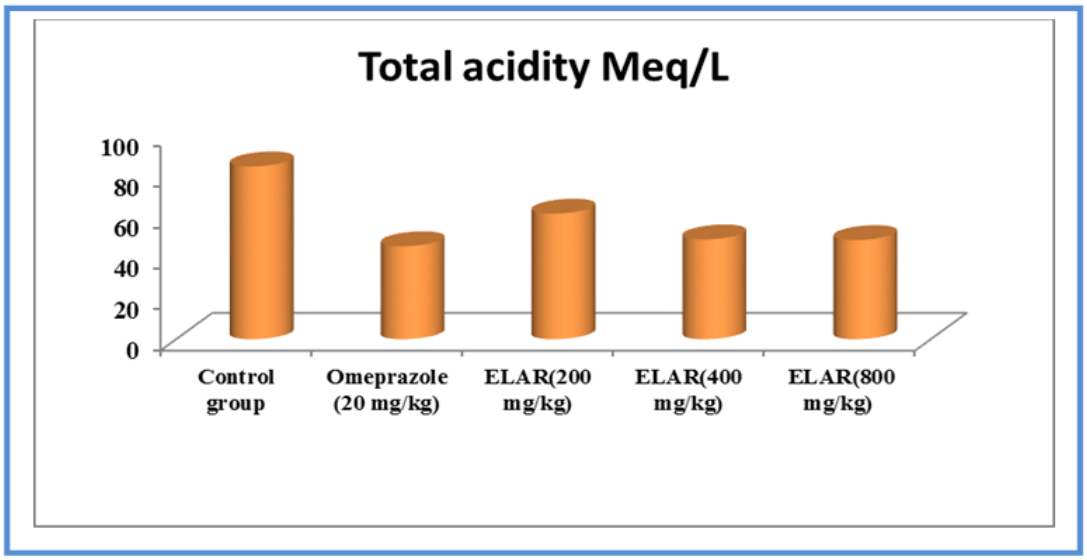
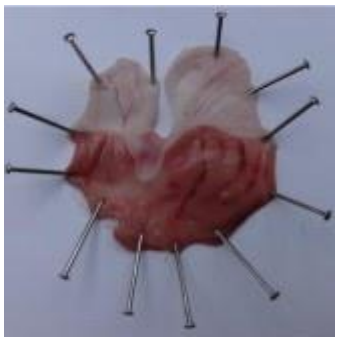


Figure 09: Bar chart of Total Acidity of Gastric Juice.

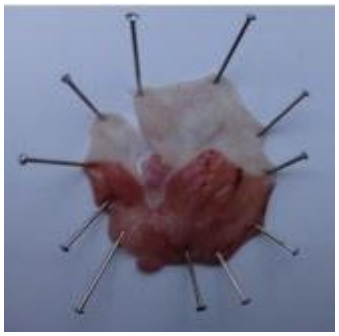
Total acidity has relation with amount of HCl present in the gastric fluid and responsible for gastric environmental because it is inversely proportional to the gastric pH. The volume of gastric juice in stomach is also significantly reduced by the standard drug (omeprazole) and ELAR dose dependent manner.

Morphology of Stomach in Ethanol Induced Ulcer

Ethanol Induced Control Group

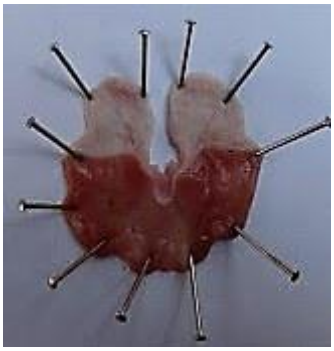


Low Dose 200 mg/kg



Medium Dose (400 mg/kg)

High Dose (800 mg/kg)



Standard Dose (Omeprazole 20mg/kg)

5. DISCUSSION

The main cause of gastric ulcer is destruction of the gastric mucosal barrier which consists of the surface epithelium and mucosal coat. This destruction may be due to either, an increase in gastric acid secretion, a decrease in mucus production or a decrease in mucosal blood flow. It is generally accepted that gastric ulcers results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism.

Prostaglandins (PG) offer protection to duodenum through both increases in mucosal resistance as well as decrease in aggressive factors, mainly acid and pepsin.

Ethanol induced gastric ulcers have been widely used for the evaluation of gastro protective activity. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. The incidence of ethanol induced ulcers is predominant in the glandular part of stomach. It was reported to stimulate the formation of leukotriene C_4 (LTC_4), mast cell secretory products and reactive oxygen species resulting in the damage of rat gastric mucosa. It has been found that oxygen derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing these ulcer. Despite the availability of many pharmaceutical products for the treatment of gastric ulcers in the market as mentioned above, their successes were limited by presence of several adverse effects (e.g. anaphylaxis reactions, gynecomastia, hematopoietic changes, thrombocytopenia, acute interstitial nephritis, nephrotoxicity & hepatotoxicity).

Due to the reported side effects of available antiulcer drugs, focused have been shifted towards natural products as the new sources of antiulcer agents. With the increasingly growing interest in natural medicine, various plants have been studied based on the traditional

knowledge of their pharmacological properties and confirmed to be useful in treating and managing ulcer. Further more, medicinal plants have been known to be amongst the most attractive sources of new drugs, and have been shown to give promising results in treatment of various diseases including gastric and duodenal ulcers.

Different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion, or to stimulate the mucosal defense mechanism. Mucosal defense mechanism by increasing the mucus production protecting the surface epithelial cells, or interfering with the PG synthesis.

This study investigated the inhibitory effects of *Asparagus racemosus* leaves extract on gastric ulcer formation induced by ethanol, compared to omeprazole, a drug whose ulcer healing effects have been extensively studied, and to an ulcer control group (vehicle). The *Asparagus racemosus* leaves extract (800 mg/kg) was found to have a protective effect on the gastric mucosa similar to that of omeprazole (20mg/kg). Omeprazole and *Asparagus racemosus* leaves extract were both found tobe protective in comparison to control group (Vehicle). This suggests that *Asparagus racemosus* leaves extract indeed has a significantantly-ulcer effect.

The results show that the *Asparagus racemosus* leaves extract is capable of providing prophylactic anti-ulcer effects against an irritant substance. The *Asparagus racemosus* leaves extract is capable of inhibiting lesion formation induced by ethanol. The accompanying significant dose-dependent decrease in Total Acidity as a dose of the drug is increased, suggests that the decrease in total acidity mechanism contributes to the acid neutralizing effect of the *Asparagus racemosus* leaves extract. It is evident from the decrease in total acidity that decrease in total acidity must have largely contributed to preventive effect of the *Asparagus racemosus* leaves extract. The Total Acidity of the gastric Content is thought to play an important role as a defensive factor against gastro-intestinal damage. This suggests that gastro-protective effect of *Asparagus racemosus* leaves extract may be mediated partly by preservation of gastric mucosal damage against acidic gastric content. This may play significant role in reducing peptic ulcer.

Absolute alcohol get metabolized in to body, leading to increased Oxygen free radicals into the gastric mucosa. The free radicals produced cause lipid peroxidation leading to membrane fluidity. Which in turn increase the influx of ca^{+} ions and result in the reduced membrane

integrity of surface epithelial cells, there by generating gastric ulcers. Free radicals have been demonstrated to be a contributing factor in tissue injury and in the modulation of pain.

The incidence of ethanol induce ulcers, predominant in the glandular part of the stomach has been reported to stimulate the form of leaukotrine C_4(LTC_4), mast Cell secretory product and reactive oxygen species resulting in the damage of rat gastric mucosa. Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents. Antioxidants could help to protect cells from damage caused by oxidative stress while enhancing the body's defense systems against degenerative diseases. Administration of antioxidants inhibits ethanol-induced gastric injury in rat.

Oxygen free radicals stimulates the proton pump, these stimulated pump then increases the secretion of acid and pepsins, which leads to increase in acidity, and thus produces ulcer. These reactive species are highly cytotoxic and can induce tissue damage.

In Ethanol induced gastric ulcers, the lesions were characterized by multiple haemorrhage red bands of different sizes along the longitudinal axis of the glandular stomach. This model is extensively used to screen drugs for cyto-protection. This study provided a substantial evidence for anti-ulcer and anti-secretory effects of an Ethanolic extract of *Asparagus racemosus* leaves. *Asparagus racemosus* leaves significantly inhibited the ulcerative lesions in all animals treated with necrotizing agents, which was further confirmed by histological findings in which Total Acidity, pH and ulcers were abolished in rats pre-treated with *Asparagus racemosus* leaves roots extract. The ability of gastric mucosa to resist injury by endogenous secretions (acid, pepsin and bile) and ingested irritant (ethanol), can be attributed to a number of factors that have been referred to collectively as mucosal defense. The gastric mucosal lesions induce by necrotizing agents such as strong alkalis are due to depression of the gastric defensive mechanisms.

6. CONCLUSION

In conclusion it appears that *Asparagus racemosus* Leaves extract may significantly decrease the acid secretion in the gastric chamber and simultaneously protect the gastric mucosa against ethanol-induced injury. *Asparagus racemosus* Leaves extracts may be exhibits an antiulcer potential activity through at least one or more possible mechanisms. Such protection was shown to be dose-dependent an as certained by the reduction or inhibition of ulcerated

areas in the gastric wall as well as the reduction or inhibition of total acidity and pH of gastric content. Protection was most prominent at a dose of 800 mg/kg of the ethanolic leaves extract of plant drug substances.

The anti-ulcer activity is probably due to the presence of bioactive compounds like flavonoids, steroids, carbohydrate, tannin, protein & amino acid, saponin and phenol. Further studies are required to confirm the exact mechanism underlying for the ulcer healing and protecting property of the extract.

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8. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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