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# EXTRACTION AND PHYTOCHEMICAL CHARACTERIZATION OF *AEGLE MARMELLOS* LEAVES AND THEIR EVALUATION FOR ANTIULCER ACTIVITIES OF ALBINO RATS

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**ABSTRACT:** In this study ethanolic extract of *Aegle marmelos* was evaluated for its anti-ulcer activity against aspirin-induced gastric ulcer in albino rats. Various biochemical parameters such as gastric volume, pH of gastric content, free acidity and total acidity, total carbohydrate content such as total protein, hexoses, hexosamine, fucose and sialic acid were estimated in alcoholic precipitate of gastric juice were examined on the test and control group of animals. The extract concentration of 250 and 500 mg/kg exhibited a protective effect on ulcer-induced models in a dose dependent manner and was comparable with the standard drugs Lansprazole. The extract of *Aegle marmelos* showed significant reduction in gastric volume, free and total acidity and ulcer index. The extracts possess significant gastro protective activity as compared with standard drug. The present study revealed that the extract of *Aegle marmelos* leaves had ulcer protective activity comparable with standard drugs Lansprazole, which may be mediated by its antioxidant effects.

**KEYWORDS:** Gastric ulceration, *Aegle marmelos*, Lansoprazole, Biochemical parameters

### I. INTRODUCTION

Peptic ulcer is a chronic, non-malignant inflammatory disease characterized by ulceration in the upper gastrointestinal tract (stomach and duodenum) where parietal cells are found. The aetiology of gastric ulceration is multifactorial and not clearly defined, but some predisposing factors have been implicated. This include duration of starvation, nature of food ingested, bile reflux [1], lessened mucosal resistance [2], alteration of gastric mucosal blood flow [3], disruption of gastric mucosal barrier by stress [4], decrease in alkaline mucosal bicarbonate and mucus secretion [5], over dosage and or prolonged administration of non-steroidal anti-inflammatory drugs [6], persistent infection with *Helicobacter pylori* [7], Zollinger-Ellison syndrome [8] and genetic factors as suggested by a higher incidence of duodenal ulcers in patients with positive family history of this disorder or blood type O [9].

Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, *H.pylori* and non-steroidal anti-inflammatory agents) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). Oxidative stress-induced tissue damage with reactive oxygen species (ROS) is implicated as a cause and consequence of a variety of disorders, including coronary heart disease, neurodegenerative disorders, autoimmune pathologies, cancer and apoptosis [10]. Exposure of gastric mucosa to damaging factors such as ethanol, thermal stress or various irritants that are commonly named 'breakers' of gastric mucosal barrier produces pathological changes [11].

For over a century, peptic ulcer disease has been one of the leading causes of gastrointestinal surgery, with high morbidity and mortality rates. The prevalence of gastrointestinal ulcers differs around the world: duodenal ulcers are dominant in the Western populations and gastric ulcers are more frequent in Asia, especially in Japan. As the prevalence of this disease increases over time, one would expect peptic ulcers to continue to have a significant global impact in the basic health systems and in patients' life quality. In recent years, gastric ulcer has also been associated with infection of gastrointestinal mucosal tissue by *Helicobacter pylori*. About 70% of patients with peptic ulcer disease are infected by *Helicobacter pylori* and eradication of this microorganism seems to be curative for this disease.

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Gastroduodenal ulcer is a common disorder of the gastrointestinal tract [12]. It is now considered that gastroduodenal ulcer is a disease of multifactorial origin but its detailed etiology is still not clear. Development of oxidative stress [13-15], lowering of gastroprotection, decrement of mucosal blood flow, delayed restitution and regeneration etc. play dominant role in the pathogenesis of ulcer [16-17]. Ulcer develops when there is imbalance between the defensive and aggressive factors on the mucosa resulting from either potentiation of aggressive factors and/or lowering of mucosal production [18]. Stress, non-steroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter pylori* are the most common causes of ulceration [13]. Cigarette smoking and alcohol ingestion are other inducers of this disease [19]. Current medicinal therapy with proton pump inhibitors and selective H<sub>2</sub> receptor blockers can efficiently cure ulcers. But none of these are devoid of side effects and execute their action within a limit. Moreover, the recurrence of ulcer after stopping the medication is very high. About 70% of ulcers could recur after stopping medication. These drawbacks of the currently available antiulcer medicines necessitate the development of newer generation phyto-genic drugs.

Many Indian medicinal plants like turmeric and neem have already shown antiulcer activity and various active compounds have been isolated from these plants [13,20]. Bael is another Indian indigenous plant which also has prominent gastroprotective effect. Pretreatment of rats with unripe bael fruit extract produced a significant inhibition of absolute ethanol induced gastric mucosal damage [21]. This activity may be due to the compound luvangetin present in the fruit. Gastric ulcer is basically mediated by the development of oxidative stress and the compounds preventing ulcer formation may act through inhibition of oxidative stress in the gastroduodenal mucosa. The phenolic compounds are potent antioxidants and have powerful antiulcer activities [5]. These compounds contain an OH group linked with the aromatic ring and thus may possess potent antioxidant and antiulcer activities.

In recent years, a large advance in chemical and pharmacological studies has contributed to the knowledge about new therapeutically active compounds obtained from the natural products [22]. These compounds can be used directly as leads for the development of new medicines or as pharmacological tools to discover new active compounds, so they can be life-saving or determine the quality of life in long-lasting diseases [23-24]. However, the incorrect use of the natural products offers dangers to society, so it is important to identify the active compounds, linking its structure with the biological activity and report the correct manner to use them with regards to dose, route of administration and frequency of use [25].

The natural active compounds classes or secondary metabolites as alkaloids, flavonoids, terpenoids, tannins and others have attracted researchers to investigate their chemical, toxicological and pharmacological features. The alkaloids represent a group of natural products that has had a major impact throughout history on the economic, medical, political and social affairs of humans. These secondary metabolites are found in about 20 % of plant species and they classified as true alkaloids, which have nitrogen atoms in heterocyclic rings, protoalkaloids, which do not have the nitrogen atom(s) in heterocyclic rings and pseudoalkaloids, which don't derive from amino acids but may have nitrogen atoms in heterocyclic rings [26].

*Aegle marmelos* Correa (family Rutaceae) appears to be relevant and this plant is available in India, Bangladesh, Myanmar, Burma and Sri Lanka. The leaves, roots, bark, seeds and fruits are edible and have medicinal values. Its distribution is mainly within the sub-Himalayan forests, in dry hilly places ascending to 4,000 feet. It is called "Shivadume", the tree of Shiva. In Hindu mythology leaves and wood of *Aegle marmelos* are used to worship Lord Shiva. The medicinal properties of this plant have been described in the Ayurveda. In fact, as per Charaka (1500 B.C) no drug has been longer or better known or appreciated by the inhabitants of India than the Bael. Hindus also believe that goddess Lakshmi resides in Bael leaves. It is therefore widely cultivated and commonly found in the vicinity of temples [27].

*Aegle marmelos* belongs to the family Rutaceae, commonly called as Bael (English), Vilvam (Tamil) and is found throughout India. Bael is a medium sized deciduous tree bearing strong axillary thorns and leaves with 3 or 5 leaflets. Bael leaves are extremely useful for treating diabetes, jaundice, cholera and asthma. Bael leaves are made into a poultice and used in the treatments of ophthalmic. Bael leaf poultice is applied to inflammations—with black pepper for edema, constipation and jaundice.

The decoction of the leaf and root bark is useful in intermittent fever, hypo-chondriasis, melancholia and palpitation of the heart [28]. The leaves and bark have been used in medicated enema. The leaves are also used in diabetes mellitus. The greatest medicinal value, however, has been attributed to its fruit [29] and the unripe fruit is said to be an excellent remedy for diarrhoea and is especially useful in chronic diarrhoeas. The effectiveness of *A. marmelos* fruit in diarrhoea and dysentery has resulted in its entry into the British Pharmacopoeia. Moreover, Chopra [30] has appropriately stated that "No drug has been longer and better known nor more appreciated by the inhabitants of India than the Bael fruit."

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Charaka has described this plant as a Rasayana. Therefore, in the present study was aimed to investigate the ulcer protective effects of ethanolic extract of dried leaves of *A. marmelos* by estimating various biochemical parameters of ulcer-induced models.

## II. MATERIALS AND METHODS

For the present study, the mature green leaves of *Aegle marmelos* belongs to family Fabaceae were collected from in and around area of Pattukkottai, Thanjavur District, Tamil Nadu, South India. The plant was identified with the help of manual of Tamil Nadu and Karnatic flora [31-32] with standard references [33].

### A. PREPARATION OF PLANT EXTRACT

The *Aegle marmelos* was collected, washed, cut into small pieces and dried at room temperature ( $28 \pm 1^\circ\text{C}$ ) for two weeks and made into powder for further analysis. Extraction is a process, to separate or isolate the secondary metabolites from plant material. It is basically two types i.e. heat and cold extraction. Heat extraction has some advantage over cold extraction like time consistency and also no contamination by microbes. An apparatus called soxhlet did heat extraction. 100g of the plant leaf powder were packed into the thimble of a soxhlet apparatus. The ratio of the plant powder and solvents were maintained at 1:4.

### B. EXPERIMENTAL ANIMALS

Adult Wistar albino rats weighing of 200 - 220 gm breed in the Central Animal House, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Trichy - 21, were used in this study. They were housed in Tarson's polypropylene cages with metal grill tops and provided with food and water *ad libitum*. They were maintained in a controlled environment under standard conditions of temperature and humidity with alternating light/dark (LD 12:12) cycle. In the laboratory, rats were fed with standard rat pellet diet (Lipton India Ltd., Bangalore). The animals used in the present study were approved by the ethical committee, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Trichy - 21, India (The Registration Number is CPCSEA/265) and were in accordance with the guidelines of the National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad, India.

### C. ACUTE TOXICITY STUDIES

Acute oral toxicity [34] study was performed as per OECD-404 guidelines (1987). 10 rats/group (5 males and 5 females) were used for the study. Group 1 was control and other three groups were treated with alcoholic extract of *Aegle marmelos* at different doses (500, 2000 and 4000 mg/kg body weight). Single dose of the extract was administered orally to each animal. Signs of toxicity, body weight, feed and water consumption of each animal was observed every day for 15 days.

### D. ANTI-ULCER ACTIVITY

The experimental procedures of Shay et al. [35] methods were used. The rats weighing 200 – 220g were divided into groups of six animals each and were placed in cages with grating floor to avoid coprophagy and fasted for 48 hours allowing free access to water. Group One Vehicle treated control (feed and distilled water). Group two treated for ulcer was induced by oral dose of Aspirin 200mg/kg of body weight once in every two days along with water. The group 3&4 treated with aspirin treated at a dose of 200mg/kg of body weight once in every two days along with the water. Then the animals were treated with the alcoholic extract *Aegle marmelos* leaves daily for 7 days in physiological saline at concentration of 250 & 500mg/kg of body weight. The group 5 Aspirin treated at a dose of 200mg/kg of body weight once in every two days along with the water. Then the animals were treated with the Lansoprasole in a dose of 8mg/kg for 7 days. Aspirin plus pylorus ligation (PL) model: Ulceration in rats was induced as described by Sathish et al. [36]. Aspirin suspension in 1% carboxy methyl cellulose in water was administered orally in a dose of 500 mg/kg once daily for three days. On eight day, pylorus was ligated as per the method of Vinothapooshan and Sundar [37]. Under light ether anesthesia, the abdomen was opened by a small mid-incision below the xiphoid process, pyloric portion of stomach was slightly lifted out and legated avoiding traction to the pylorus or damage to its blood supply.

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The stomach was replaced carefully and the abdomen wall closed by interrupted sutures. The *Aegle marmelos* were administered once daily orally for seven days. The animals were deprived of both food and water during the post-operative period. Four hours later the rats were sacrificed by using anaesthetic ether and their stomachs were dissected. Ulceration in the stomach was assessed by means of a scoring technique whereby macroscopic examination of the stomach was made using a hand lens and ulcers were scored using the method and criteria of Vivek Sharma and Ranjani [38]. Normal gastric mucosa was scored nil, punctate haemorrhage, pinpoint ulcer was scored 0.5, one or two small hemorrhages ulcer was scored 1.0 while ulcers greater than 3mm in diameters were scored 2.0 ulcer index and percentage inhibition of ulceration was calculated.

$$\text{Ulcer index} = \frac{(\text{Mean degree of ulceration} \times \% \text{ group of ulceration})}{100}$$

$$\% \text{ inhibition of ulceration} = \frac{(\text{Ulcer index in control} - \text{Ulcer index in test})}{\text{Ulcer index in control}} \times 100$$

## E.COLLECTION OF GASTRIC JUICE

The stomach was excised carefully keeping the esophagus closed, opened along the greater curvature and the luminal contents were removed as described [33]. The gastric contents were collected in a beaker and centrifuged at 1000 rpm for 10 minutes as recommended. Gastric juice was collected from the pylorus-ligated rats. The gastric juice thus collected was centrifuged and the volume of gastric juice as well pH of gastric juice was measured. The sodium ( $\text{Na}^+$ ) and potassium ( $\text{K}^+$ ) ion concentration of gastric juice was carried out in flame photometer [39].

Biochemical estimation in aspirin plus induced ulcer models the following were estimated by procedures described gastric volume [40], pH of gastric content [41], free acidity and total acidity [42]. The total protein estimated [43], the total carbohydrate such as hesoses, hexosamine, fucose, sialic acid [44].

## III. RESULTS AND DISCUSSION

The present study was carried out to evaluate the antiulcer activity of *Aegle marmelos* against aspirin induced toxicity in albino rats. The effectiveness of this medicinal plant was screened by assessing biochemical changes of different groups of experimental animals. The results were expressed as mean  $\pm$  standard deviation. The result of phytochemical screening of the aqueous and alcoholic extracts of *Aegle marmelos* revealed the presence of alkaloids, flavanoids, phytosterols, tannins and phenols. The plant extract of *Aegle marmelos* used for the present work was choosing on the basis of their medicinal values. The natural plant parts are having a wide range of medicinal properties like hepatoproduative, antimicrobial, diuretic, emollient, febrifuge, narcotic, purgative and sedative. Previous study in the naturally the ethanolic extracts of *Aegle marmelos* were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids, and steriods, but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results [45].

The phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins [46]. The present study carried out on the plant samples revealed the presence of medicinally active constituents. It is quite possible that some of the plants that were ineffective in this study do not possess antibiotic properties, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in methanol or water. The drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants [47].

*Aegle marmelos* possessed very high levels of flavonoids and are employed in medicinal uses. The plants studied here can be seen as a potential source of useful drugs. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. The antiulcer activities of these plants for the treatments of the diseases as claimed by traditional healers are also being investigated. There is balance between the aggressive and the mucosal protective factors in stomach. Thus drug therapy of peptic ulcer has been commonly

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targeted at either counteracting the aggressive factor or stimulating defensive ones. Despite the progress in conventional chemistry and pharmacology in producing highly effective drugs, some of them are expensive and have different adverse effects [48], however screening plants for active drugs is still important and might provide a useful source of new antiulcer compounds for developing pharmaceutical drugs or alternatively as simple dietary adjuncts to existing therapies.

In aspirin plus pylorus ligation model, ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity. In control animals aspirin plus pylorus ligation increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting in higher ulcer index. Although in most of the cases the aetiology of the ulcers is unknown, it is generally accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanisms [12, 49-50]. To regain the balance, different therapeutic agents including plant extracts are used. *Aegle marmelos* extract is one such herbal drug undertaken for the present study primarily to evaluate its antiulcerogenic potential. The number of lesions in the untreated ulcer group was quite high and among the treated groups, the group pretreated for study periods had a dramatic decrease in the number of lesions. The numbers of lesions present on the gastric mucosa are indicative of the ulcer severity. A significant reduction in the number of lesions in the pretreated *Garcinia cambogia* groups may be due to the appetite suppressant effect of the drug, thereby inhibiting gastric acid secretion, an important factor in ulcer formation [51].

In aspirin plus pylorus ligation induced gastric ulcer model the ethanol extracts of *Aegle marmelos* reduced the gastric volume, free acidity, total acidity and ulcer index thus showing the anti-secretory mechanism involved in the extracts for their anti-ulcerogenic activity. Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity. In case of vehicle control, aspirin plus pylorus ligation increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting into increase in ulcer index [12, 52]. Aspirin induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Aspirin induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [53]. The extract shows protection against characteristic lesions produced by Aspirin administration this antiulcer effect of *Aegle marmelos* may be due to both reductions in gastric acid secretion and gastric cytoprotection.

Ramamurthy and Selvarani [12] reported that the acid secretory parameters such as pH, gastric volume, free acidity and total acidity were increased significantly in the aspirin administered group. Administration of ethanolic extracts of *Azima tetracantha* exhibited a significant reduction in all the parameters and the results were comparable with the standard drug Lansoprazole 8 mg/kg. Determination of the concentrations of various muco-proteins such as total protein, total hexoses, hexosamine, fucose and sialic acid revealed a decrease in ulcer induced group. The present study *Aegle marmelos* showed significant dose-dependent ulcer protective effect against aspirin plus pylorus ligation induced gastric ulcers. *Aegle marmelos* used in the study have been found to be effective against aspirin pylorus ligation model. It is evident from the results that these drugs produce reduction in the intensity of gastric ulceration as observed from reduced ulcer index in the drug treated groups. However, inconsistent results were obtained as regards to other parameters such as volume of gastric acid secretion, free and total acidity and pepsin activity.

Table 5 indicates the level of total carbohydrate such as hexoses, hexosamine, fucose and sialic acid in experimental rats. Group Aspirin induced group shows decreased level of total carbohydrates when compared to normal control and *Aegle marmelos* leaves treated groups. Aspirin induced ulcer study was not able to show a uniform rise in individual carbohydrate content but, the total carbohydrate content of gastric juice was significantly increased in the presence of alcoholic extracts of *Aegle marmelos* leaves and lansoprazole treated groups. Our study has established various causes responsible for the development of ulcers due to ulcer induced model such as increased metabolism of carbohydrates, increased synthesis of nucleic acids and also exhaustion of carbohydrates and other compensatory mechanisms. Relative to the normal levels the hexose, hexosamine and sialic acid content of the gastric juice decreased considerably in the ulcer group while the protein level increased. The increase in protein content of the gastric juice indicates damage to the gastric mucosa as a result of which plasma protein leaks into the gastric juice [54]. The decrease in the glycoprotein moieties in the gastric juice may be attributed to the decreased activity of defence mechanisms as a result of damage to the gastric mucosa. In other words disintegration and degradation of glycoprotein moieties into their

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simpler components in the process of Aspirin induced injury might have resulted in minimal quantities of glycoprotein in the gastric juice. The levels of protein, hexose and hexosamine were maintained at near normal levels in the group pretreated with *A. marmelos*. *Cassia auriculata* inhibits vagus nerve stimulation, thereby reducing the HCl output and acidity. Being an effective appetite suppressor [51], it protects the quantity and quality of mucus secretion against the offensive assault of acid. *Aegle marmelos* appears to regulate both acid output and mucus secretion.

It has been proposed that aspirin induces gastric ulceration by reducing the gastric mucus which protects the gastric mucosa from acid and pepsin and by weakening the gastric mucosal barrier. Muthusamy et al. [55] determined that the hexosamine, one of the major components of mucus, in the tissues of the corpus and antrum. Our results agree with other reports [56-57] that aspirin-induced ulceration correlates with the reduction in the hexosamine level of gastric tissue. Although the hexosamine level fell to a low level abruptly after aspirin administration, ulceration did not develop until several hours after the treatment. This observation suggests that the low hexosamine level is a cause of ulceration and not a result that the visual ulcer is exerted by acid and/or pepsin after the gastric mucus has been reduced by aspirin. The reduced hexosamine level in the tissues of both the corpus and antrum was restored to the intact level by pretreatment with clotiazepam. The ulcer index, ulcer inhibition shows that the maximum inhibition of ulcers in this study was of Lansprazole (86%). The ulcer inhibition of *Aegle marmelos* leaves extract (500mg/kg) (84%) was better. *Aegle marmelos* leaves extract (250mg/kg) had the least ulcer inhibition effect (67%). From this study, it is clear that *Aegle marmelos* leaf extract have significant anti-ulcer activity in animal models. It has muco-protective activity and gastric anti-secretory when compared with that of reference drug Lansprazole. The extract is non-toxic even at relatively high concentrations. The anti-ulcer activity is probably due to the presence of flavonoids.

**Table 1. Qualitative Phytochemical screening of *Aegle marmelos***

S. No	Name of Test	Test applied / Reagent used	Aqueous extract	Alcohol extract
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D] ) Dragendorff's test	+	+
2	Flavanoids	HCl and magnesium turnings	+	+
3	Carbohydrate	Molisch's test	+	+
4	Tannins & Phenols	A] 10% Lead acetate B] FeCl <sub>3</sub>	+	+
5	Test for Steroids	A] Salkowski's Test B] Libermann-Burchard's Test	+	+
6	Gums & Mucilages	Alcoholic Precipitation	-	-
7	Fixed oil & Fats	Spot test	+	+
8	Saponins	Foam test	-	-
9	Phytosterols	LB test	+	+
10	Volatile oils	Hydro distillation method	+	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C] Xanthoprotein test	+	+

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**Table 2. Effect of *Aegle marmelos* on aspirin induced ulcer index lesions, pH and protein in rats.**

S. No	Treatment group	Ulcer index	pH	Protein ( $\mu\text{g/dl}$ )
1	Control	$1.25 \pm 1.22$	$5.2 \pm 1.02$	$310 \pm 2.85$
2	Ulcer control (Aspirin 200mg/kg)	$3.50 \pm 1.31$	$2.9 \pm 1.32$	$482 \pm 2.25$
3	<i>Aegle marmelos</i> (500 mg/kg)	$2.75 \pm 1.03$	$4.8 \pm 1.06$	$335 \pm 2.30$
4	<i>Aegle marmelos</i> (250 mg/kg)	$2.15 \pm 1.21$	$4.5 \pm 1.03$	$378 \pm 2.29$
5	Lansprazole (8 mg/ kg p.o.)	$1.63 \pm 1.06$	$4.7 \pm 1.65$	$315 \pm 2.75$

**Table 3. Effect of *Aegle marmelos* on sodium and potassium concentration in aspirin induced rats.**

S. No	Treatment group	Sodium (mEq/L)	Potassium (mEq/L)
1	Saline Control	$135 \pm 1.22$	$4.5 \pm 2.13$
2	Ulcer control (Aspirin 200mg/kg)	$85 \pm 1.92$	$12.1 \pm 2.84$
3	<i>Aegle marmelos</i> (500 mg/kg)	$98 \pm 1.17$	$6.2 \pm 2.32$
4	<i>Aegle marmelos</i> (250 mg/kg)	$115 \pm 1.15$	$7.5 \pm 2.42$
5	Lansprazole (8 mg/ kg p.o.)	$97 \pm 1.24$	$5.5 \pm 2.15$

**Table 4. Effect of *Aegle marmelos* on gastric secretion, acidity and pepsin activity in aspirin induced rats.**

S. No	Treatment group	Volume of gastric juice (mEq/L)	Free acidity (mEq/L)	Total acidity (mEq/L)	Pepsin activity ( $\mu\text{g/ml}$ )
1	Saline Control	$105 \pm 1.14$	$10 \pm 2.3$	$65 \pm 1.5$	$9.2 \pm 0.15$
2	Ulcer control (Aspirin 200mg/kg)	$156 \pm 1.22$	$17 \pm 2.9$	$45 \pm 1.7$	$5.1 \pm 0.24$
3	<i>Aegle marmelos</i> (500 mg/kg)	$123 \pm 1.07$	$13 \pm 2.7$	$59 \pm 1.2$	$7.3 \pm 0.41$
4	<i>Aegle marmelos</i> (250 mg/kg)	$129 \pm 1.42$	$15 \pm 2.5$	$53 \pm 1.3$	$6.5 \pm 0.22$
5	Lansoprazole (8 mg/ kg p.o.)	$115 \pm 1.09$	$12 \pm 2.8$	$61 \pm 1.8$	$8.4 \pm 0.45$

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**Table 5. Effect of *Aegle marmelos* on gastric secretion in aspirin induced rats. Effect on carbohydrates.**

Treatment group	Carbohydrate ( $\mu\text{g/ ml}$ )				Total Carbohydrate
	Hexoses	Hexosamine	Fucose	Sialic acid	
Saline Control	78.2 $\pm$ 2.5	25.4 $\pm$ 1.6	125.7 $\pm$ 1.8	21.5 $\pm$ 1.5	250.8 $\pm$ 12.7
Ulcer control (Aspirin 200mg/kg)	59.5 $\pm$ 2.1	11.5 $\pm$ 1.3	80.75 $\pm$ 1.8	10.2 $\pm$ 1.7	161.9 $\pm$ 15.5
<i>Aegle marmelos</i> (500 mg/kg)	65.7 $\pm$ 2.2	16.7 $\pm$ 1.7	115.6 $\pm$ 1.6	19.8 $\pm$ 1.8	217.8 $\pm$ 18.9
<i>Aegle marmelos</i> (250 mg/kg)	64.5 $\pm$ 2.5	16.1 $\pm$ 1.7	118.2 $\pm$ 1.8	18.3 $\pm$ 1.2	217.1 $\pm$ 17.1
Lansprazole (8 mg/ kg p.o.)	73.8 $\pm$ 2.6	14.5 $\pm$ 1.5	117.8 $\pm$ 1.2	18.8 $\pm$ 1.6	224.9 $\pm$ 19.5

## IV. CONCLUSION

In rats with the gastric ulcer treatment with *A. marmelos* shows decrease in gastric mucosa and biochemical level with extremely significant, while there is an improvement in ulcer. The powder of *A. marmelos* shows potent antiulcer activity. From this study, it is clear that *A. marmelos* leaf extract have significant antiulcer activity in rats. It has mucoprotective activity and gastric anti-secretory when compared with that of reference drug lansoprazole. The extract is non-toxic even at relatively high concentrations. The anti-ulcer activity is probably due to the presence of flavanoids. Thus always have in mind that "Prevention is better than cure".

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