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Antiulcer Activity of Clerodendrum Serratum Extract on Albino Rats

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ABSTRACT

Clerodendrum serratum (Verbenaceae) is an important medicinal plant growing in the tropical and warm temperate regions like Africa, Southern Asia; Malaysia and distributed throughout in forests of India and Sri Lanka. It is traditionally valued and reported for treating pain, inflammation, rheumatism, respiratory disorders, fever and malarial fever in India with a long history.Ethnomedicinal uses *C. serratum* (Bharangi) is widely used in indigenous systems of medicine for the treatment of respiratory disease, especially asthma, and several other diseases in a crude combination with several other drugs According to Aryabhishek it is useful in cough, swelling, breathlessness, wound, fever, rheumatism, tuberculosis etc. The Ayurvedic Pharmacopoeia of India indicated the use of the dried roots for cough, bronchitis, dyspnea, chest diseases and sinusitis. Pharmacognostic evaluation established the macroscopic and microscopic parameters for the identification of whole plant and its powder. Physicochemical parameters were also set forth while quantitative phytochemical analysis showed that the ethyl acetate fraction had the highest quantity of phenols, flavonoids, and tannins.

The present study was carried by aspirin plus pylorus ligation induced ulcer models in albino rats. The antiulcer activity of ethanolic extract of *Clerodendrum Serratum* (EOCS) and ethyl acetate extract of *ClerodendrumSerratum* (EACS) were compared with standard drugs (Ranitidine). In pyloric ligation induced ulcer model, the studied parameters were gastric volume, pH, total acidity, free acidity, and ulcer index.In aspirin pyloric ligation model the volume of gastric content, total/free acidity and pepsin activity was significantly decreased and pH of the gastric juice was significantly increased at in EOCS treated groups as compared to control group. All the doses of EOCS showed dose dependent antiulcer effect as well as significant ($p<0.05$) reduction in the ulcer index as compared to control group in all the experimental models.The results of the study indicate that the Ethanol extract of *Clerodendrum serratum* have better potential against ulcer.

Keywords: *Antiulcer effect, Pylorus ligation, Ulcer index, Clerodendrum serratum*

1. INTRODUCTION

Plants have been a major source of agents used for the management of various diseases across the world for millennia and are still of contemporary importance as 80% of people worldwide depend on herbal remedies in their primary healthcare system, and over 25% of prescribed medicines in developed countries are of plant origin [1-2]. Over the last few decades, an upsurge has been witnessed in the public acceptance of natural therapies, leading to the increased usage of herbal medicines and phytonutrients. In Europe, over 1300 medicinals are being used for various ailments, while 118 out of the top 150 prescription drugs in the USA are based on natural resources [3]. Due to the increased demand for medicinal plants and the continuous loss of natural habitat, medicinal plants are facing a serious challenge to their existence. An upsurge in the usage of herbal medicines, a decrease in natural sources, and less availability of genuine crude drugs are resulting in adulteration and substitution practices. Furthermore, crude drugs are collected, stored, distributed, and sold in raw form without any special packaging, which may also lead to adulteration and substitution [4-5].

Ulcer is a major disease of gastrointestinal system which affects 10% of the world population with different aetiologies. Chronic alcohol intake, smoking, excessive stress, chronic usage of non-steroidal anti-inflammatory drugs and H.pylori bacterial infection are the crucial causes of peptic ulcer characterized by inflammation, mucosal bleeding and abdominal pain in patients [6]. These ulcers can develop when the imbalance occurs between the gastroprotective (mucus, bicarbonate and prostaglandins) and aggressive (acid, pepsin, bile salts and Helicobacter pylori bacteria). The recent approach to peptic ulcer is managed by inhibition of gastric acid secretion, promotion of gastro-protection, blocking apoptosis and stimulation of epithelial cell proliferation for effective healing [7-8]. The conventional drugs used in the treatment of ulcer include histamine receptor antagonists, prostaglandins analogues, proton pump inhibitors, cytoprotective agents, antacids and

anticholinergics, but most of these drugs produce undesirable side effects or drug interactions and may even alter biochemical mechanisms of the body upon chronic usage. Hence, herbal medicines are generally used in such chronic cases, wherein drugs are required to be used for long periods [9].

Clerodendrum serratum (Verbenaceae) is an important medicinal plant growing in the tropical and warm temperate regions like Africa, Southern Asia; Malaysia and distributed throughout in forests of India and Sri Lanka. It is traditionally valued and reported for treating pain, inflammation, rheumatism, respiratory disorders, fever and malarial fever in India with a long history [10-11].Ethnomedicinal uses *C. serratum*(Bharangi) is widely used in indigenous systems of medicine for the treatment of respiratory disease, especially asthma, and several other diseases in a crude combination with several other drugs such as Sati (Hedychium spicatum Sm. Zingiberaceae) and Pushkarmoola (Inula racemosa Hook.f. Asteraceae) and also present in capsule or tablet form of various strengths. According to Aryabhishek it is useful in cough, swelling, breathlessness, wound, fever, rheumatism, tuberculosis etc. The Ayurvedic Pharmacopoeia of India indicated the use of the dried roots for cough, bronchitis, dyspnea, chest diseases and sinusitis [12-13]

However, there is no scientific evidence on prevention of peptic ulcers of *Clerodendrum serratum*. Hence, the present study was conducted with the aim to investigate the antiulcer activity of ethyl acetate and Ethanol Extract of *Clerodendrum serratum*.

2. MATERIALS AND METHODS

Procurement of *Clerodendrum Serratum* seed: As the crude drugs form the basis for the manufacture of wide range of medicinal preparations needed by people, the development of pharmacognostical research has become indispensable for procuring therapeutically potent medicine prepared from genuine drug material. The pharmacognosists have a serious responsibility, to take the initiative not only in correctly locating the plant mentioned in old treatises and pharmacopeias but also making them available to scientists in other disciplines to test the use for which they are acclaimed. Clerodendrum Serratum seed were procured from local market of Bhopal and authenticated by botanist.

The seeds were pulverized into small pieces, dried in sun and ground with the help of an electrical grinder to get powder, stored in airtight containers and used for phytochemical and pharmacological studies.

Preparation of Plant Extract: *Clerodendrum Serratum* seed powdered (100 g) was successively extracted with the following solvents of increasing polarity in a soxhlet apparatus. The dried powder was packed in Soxhlet apparatus extracted with 250 ml of petroleum ether for 72 h at 50ºC. After the extraction, extract filtered and solvent was removed with the help of rotatory evaporator. The same process was carried out to get ethyl acetate and ethanol extracts. The total yield of the extracts obtained after removing the solvents was calculated. All the extracts were concentrated by distilling the solvents and the extracts were dried in an oven. Each time before extracting with the next solvent, the marc was dried in an air. The completion of the extraction was confirmed by evaporating a few drops of extract from the thimble on watch glass to observe that no residue remained after evaporation of the solvent. The yield of the extract was found to be 11.87 %w/w.The preliminary phytochemical screening was performed with the ethanolic extract of *Clerodendrum Serratum* seed for the detection of various phytochemicals [14].

Selection of animals and preparation of groups: Healthy albino rats of either sex, weighing between 180-250g disease free animal were used. They were housed in standard environmental conditions of temperature, humidity, and light and provided with standard rodent food and water ad libitum as per IEAC guideline (IEAC/918/CPCSEA/5).

Acute Oral Toxicity Study: Healthy adult male albino rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the ethanol and ethyl acetate extract of *Clerodendrum Serratum* seed were administered to each group of rats (Each group carries 6 rats) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hour, for any gross behavioural changes and further up to 72 hour, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425. The extract of *Clerodendrum Serratum* was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for antiulcer evaluation was 100 and 200 mg/kg respectively [15].

Animal Grouping and Treatment Schedule for Antiulcer Study: In all the experimental models, male *albino* rats were selected and divided into five groups of six animals each. Animals were fasted for 24 hour before the study, but had free access to water. Group I treated as normal control, received only normal saline; group II is Disease control; group III is standard received ranitidine 50 mg/kg (P.O.) and remaining group treated as treatment groups, received the graded dose of ethanol and ethyl acetate extract of Clerodendrum Serratumat 100 and 200 mg/kg, (P.O.) for 7 days (once in a day) respectively. Anti-ulcer Activity of extract of *Clerodendrum Serratum*were performed on ulcer induce by aspirin + Pylorus ligation induced model.

Table 1: Animal grouping for anti-ulceractivity

Group I	Normal control	Normal saline (0.85%)
Group II	Disease control	2% gum acacia solution
Group III	Standard	Ranitidine (50mg/kg b.w) in 2% gum acacia
Group IV	EACS (100 mg/kg)	Ethyl acetate extract of C. Serratum (100 mg/kg)
Group V	EACS (200 mg/kg)	Ethyl acetate extract of C. Serratum (200 mg/kg)
Group VI	EOCS (100 mg/kg)	Ethanol extract of C. Serratum(100 mg/kg)
Group VII	EOCS (100 mg/kg)	Ethanol extract of C. Serratum(200 mg/kg)

Pyloric Ligation Induced Gastric Ulceration:

Aspirin + Pyloric ligation model: Among the various methods available pyloric ligation methodhas been widely used in the screening model for gastroprotective activity. In the present work gastroprotective activity of selected plant extracts were tested against aspirin + Pylorus ligation gastric ulcer models. An decrease in the pH, increase of gastric juice content, lesions formation in the GI tract are the indices of ulcer formation. The ability of above mentioned extracts to increase the pH, decrease in the volume of gastric juice and less ulcer formation near to standard values are indication of their gastroprotective potential.

The animals were divided into seven groups, each containing six animals. Group I served as Aspirin (200 mg/kg, p.m.) +PL control. Group II received Ranitidine (50 mg/kg, p.o.) as standard drug+Aspirin+ PL. Groups III and IV and V received plant extract at the dose of 100, 200 and 300 mg/kg, p.o+PL. Groups II–V received the assigned drug treatment for the respective 10 days daily. From days 8 to 10, animals of all groups received aspirin orally as an aqueous suspension at the dose of 200 mg/kg, 2 h after the administration of the drugs. Animals in all groups were fasted for 18 h after the assigned treatment, anesthetized and the pyloric was ligated. The rats were sacrificed after 4 h by excess anesthesia (ether). The stomach was removed, opened along greater curvature and the gastric lesions were observed. The gastric ulcers were counted and the ulcer index was determined. The gastric juice was collected, centrifuged and the volume of the supernatant was expressed as mL/ 100 gm.b.wt. Free acidity and total acidity were determined by titrating with 0.01N NaOH using Topfer's reagent and phenolphthalein as indicators. The free and total acids were expressed as mEq/L. The total acid output was determined and expressed as mEq/L. Four hours after ligation, the stomach was dissected out and contents were collected into clean tubes. The volume, pH and total acid content of gastric juice were determined. The contents were centrifuged, filtered and subjected to titration for estimation of total acidity. From the supernatant, aliquots (1 ml each) were taken for the determination of pH, total or free acidity and pepsin activity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity [16-17].

The numbers of ulcers were counted and scoring of ulcer was made as follows: Normal colored stomach (0), Red coloration (0.5), Spot ulcer (1), Haemorrhagic streak (1.5), Deep ulcers (2) and Perforation (3). Mean ulcer score for each animal was expressed as ulcer index [18].

Ulcer index (UI) was measured by using following formula: $UI = UN + US + UP \times 10{-}1$

Where, UI (Ulcer Index); UN (Average number of ulcers per animal); US (Average number of severity score); UP (Percentage of animals with ulcers). The percentage inhibition of ulceration was calculated and compared with control.

Effect of ethanolic extract of Clerodendrum Serratum seeds (EOCP) and Clerodendrum Serratum fruit (EOBH) on Aspirin + Pylorus ligation induced ulcer: Aspirin+pylorus ligation-induced gastric ulcer model is a useful model to induce severe ulceration in experimental animals. Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H+ ions. The inhibition of mucosal prostaglandin production occurs rapidly following oral administration of aspirin. This is correlated with the rapid absorption of these drugs through the mucos. In pylorus ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration

Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H+ ions. In pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration. Aspirin was administered to PL rats; thus, aspirin further aggravated the acidity and the resistance of the gastric mucosa was decreased thereby causing extensive damage to the glandular regions of the stomach.

Ethanol extracts of *Clerodendrum Serratum* seeds and *Clerodendrum Serratum* fruits at a dose of 100 and 200 mg/kg b.w., were tested for gastroprotective activity using pyloric ligation rat model. Peptic ulcer is results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid.

Antiulcer study has been performed using 100 and 200 mg/kg of ethanol extract of *Clerodendrum Serratum* seeds against aspirin + Pylorus ligation gastric ulcer models. The ethanol extract were administered to various groups, orally, twice a day as described earlier. The result indicated a dose-dependent antiulcerogenic activity of extract EOCS. The best effect observed was at dose of 200 mg/kg onwards with EOCS. So for further studies on other biochemical parameters of gastric secretion or mucosal studies, a dose of 200 mg/kg was selected.

Table 2: Effect on Volume of HCl and pH by *Clerodendrum Serratum*seeds extract

Treatment	**Volume of HCl**	**pH**
Normal control	6.01±0.12	1.2±0.04
Standard	2.41±0.14	5.5±0.23
EACS (100 mg/kg)	4.89±0.14	4.5±0.35
EACS (200 mg/kg)	3.6±1.11	4.9±0.24
EOCS (100 mg/kg)	3.92±0.21	4.8±0.27
EOCS (200 mg/kg)	2.89±1.11	5.1±0.21

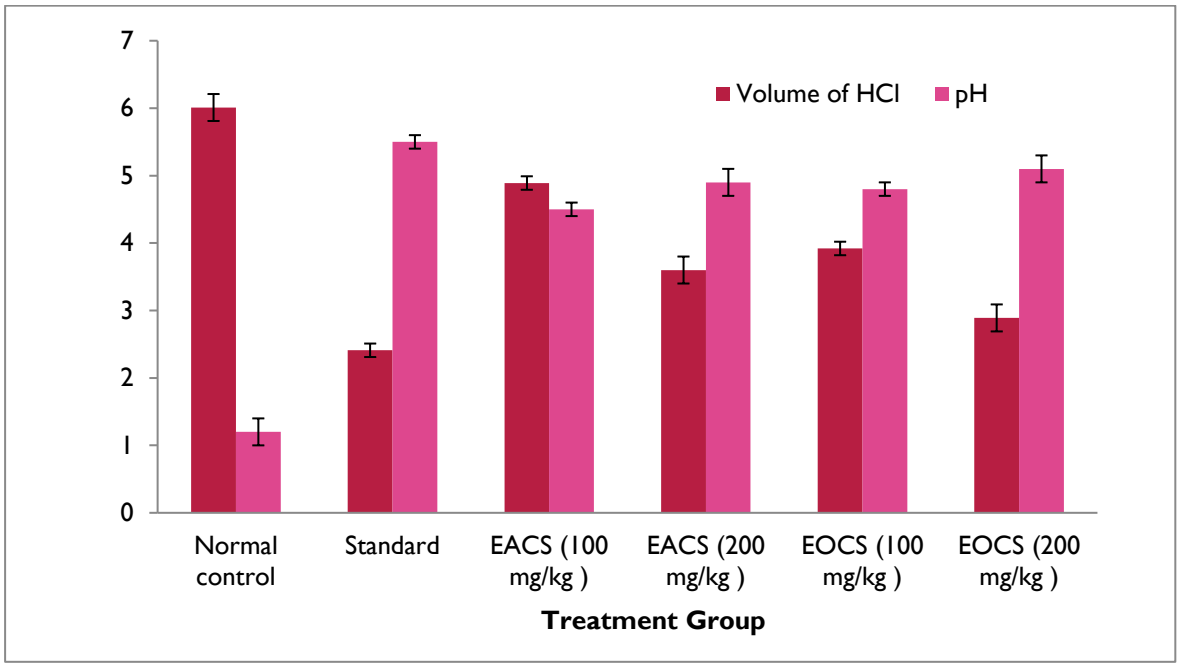

Figure 1: Effect on Volume of HCl and pH by *Clerodendrum Serratum seeds* extract

Effect on Total acidity and Free acidity As there is increase in the total acidity and free acidity content the chances of ulcer formation will also be high.

Table 3: Effect on total acidity and free acidity by *Clerodendrum Serratum*seeds extract

Treatment	Total acidity mEq/L	Free acidity mEq/L
Normal control	98.1± 2.1	63.2±4.2
Standard	31.2±1.9	20.3±1.3
EACS (100 mg/kg)	66.12±0.49	49.2±4.2
EACS (200 mg/kg)	60.23±0.42	41.1±3.4
EOCS (100 mg/kg)	54.9±0.36	31.2±0.21
EOCS (200 mg/kg)	45.5±0.35	22.1±0.19

Total acidity exhibited by the standard group was significantly less when compared to the total acidity exhibited by the control group. The standard group showed reduction in total acidity and free acidity significantly when compare with the control group. Ethanol extract of *Clerodendrum Serratum*seeds at dose of 200mg/kg showed more reduction in total acidity and free acidity compared to ethyl acetate extract.

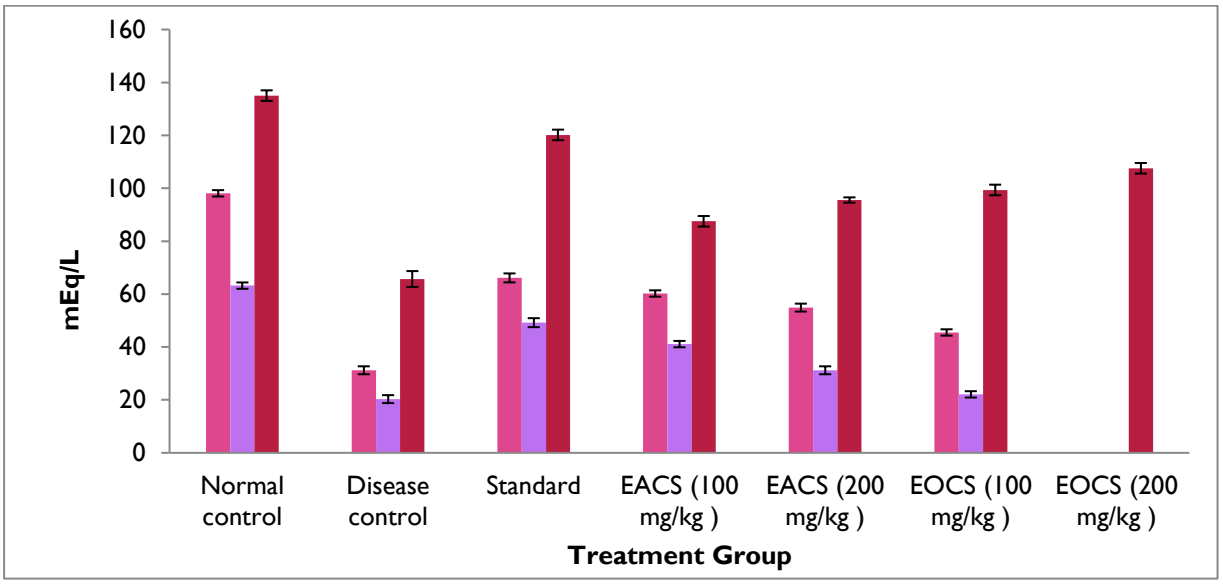

Figure 2: Effect on Total acidity and Free acidity by *Clerodendrum Serratum*seeds extract

Effect on Ulcer index and percent protection: Ulcer index is the index which indicates the severity of ulcers.

Table 4: Effect on Ulcer index and percent protection by *Clerodendrum Serratum seeds* extract

Treatment	Ulcer index	% protection
Normal control	0.67 ± 0.06	0
Standard	0.20 ±0.02	70.2 %
EACS (100 mg/kg)	0.48±0.14	28.4 %
EACS (200 mg/kg)	0.34±0.12	49.3 %

EOCS (100 mg/kg)	0.28±0.03	58.3 %
EOCS (200 mg/kg)	0.23±0.02	65.7 %

Increase in the ulcer index more is the severe condition of ulcer. Standard drug treated group showed ulcer index significantly less as compare to the ulcer index of control group. Ulcer index shown by the EOCS treated group (100, 200mg/kg b.w) was found to be less than Ulcer index shown by the EACS treated group (100, 200mg/kg b.w). Ethanol extract of *Clerodendrum Serratum*seeds at showed ulcer index compared to standard treated group.

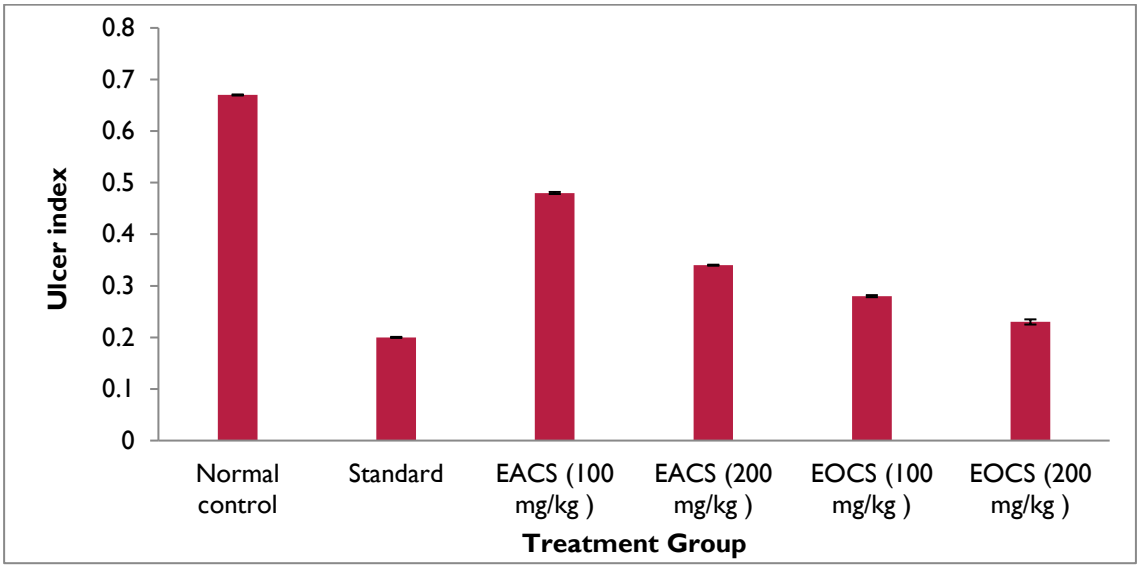

Figure 3: Effect on Ulcer index and percent protection by *Clerodendrum Serratum*seeds extract

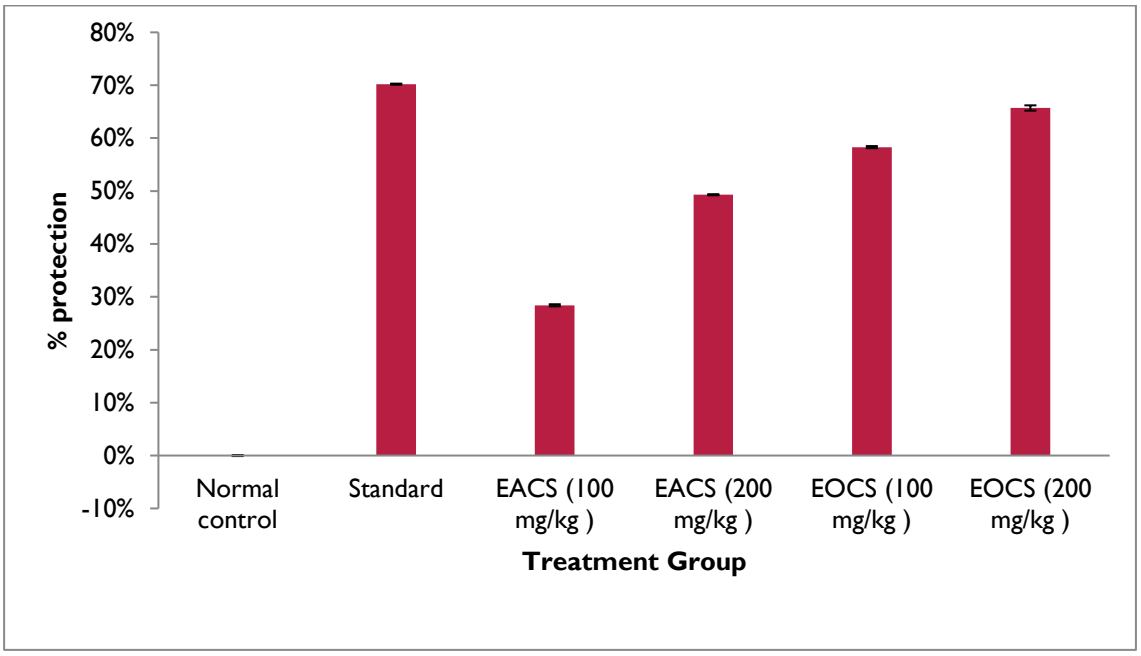

Figure 4: Effect on Ulcer index and percent protection by *Clerodendrum Serratum*seedsextract

Standard drug treated group has shown the percentage protection of 70.2%. Whereas, Ethanol extract at a dose of 100, 200mg/kg b.w has shown percentage protection of 58.3%, 65.7 % respectively. Whereas, ethyl acetate extract at a dose of 100, 200mg/kg b.w has shown percentage protection of 28.4 %, 49.3% respectively. Ethanol extract showed more percent protection than ethyl acetate extract. Ethanol extract of *Clerodendrum Serratum*seeds showedpercent protection in ulcer.

3. DISCUSSION

The cause of gastric ulcer is due to stress induced increase in gastric acid (HCl) secretion and these acid secretions promote ulceration due to exposure of the unprotected lumen of the stomach to the accumulating acid. Pylorus ligation induced ulcers are shown by auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier which resulted as upper gastrointestinal damage including lesions, ulcers and life threatening perforation and haemorrhage. The pyloric ligation of the stomach causes accumulation of gastric acid which leads to development of ulceration in stomach. The agents who decrease gastric acid secretion and increase mucus secretion are effective in preventing the ulcers induced by this method. Like ranitidine, omeprazole acts as anti-ulcer agent by antisecretory mechanism via inhibition of gastric secretion and pepsin activity. In the present study, Ethanol extract of *Clerodendrum Serratum* prevents the ulcer may be by antisecretory property.

Several scientific studies revealed that the phytoconstituents like flavonoids, tannins, terpenoids and saponin were responsible for gastro protective agents. Tannins possess as an antiulcer agent by its astringency property and vasoconstriction effects. Due to precipitation of micro proteins on the ulcer site, a protective layer was formed which hinders gut secretions and protects the mucosa from toxins and other irritants. Previous studies have recommended that these above active compounds had ability to stimulate mucus, bicarbonate and prostaglandin secretion and neutralize with the deteriorating effects of reactive oxidants in gastrointestinal lumen. Therefore *Clerodendrum Serratum* possess antiulcer activity, may be due to presence of tanins, flavonoids and terpenoids.

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