# An Experimental Approach for Antioxidant and Antiulcer Potential of *Clerodendrum serratum* Rhizome

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***Abstract-***

*The present research work is aimed at evaluating antiulcer potential of methanolic extract of Clerodendrum serratum against ethanol-induced ulcer. Clerodendrum serratum as effective treatment against asthma, body ache, cholera, eye disorder, ulcers, snakebite, wound, tuberculosis and epilepsy. In this project 36 hours fasted wistar albino rats were used for the study but had free to water. Animals were randomly divided into three groups. Group-I animals were treated as normal control and received only vehicle (distilled water, 10ml/kg, p.o). Group-II animals serve as standard control and were treated with Rabeprazole at a dose 20 mg/kg, p.o., while Group-III animals serve as test control and were dosed with 200mg/kg extracts, p.o. All the rats were treated 90% v/v ethanol at a dose 200 gm/ml orally one hour after the drug treatment. After one hour of the treatment of all drugs, animals were sacrificed with high dose of ether anaesthesia. The stomach were isolated and opened along the greater curvature and remove gastric contents and blood clots and examined by a×5 magnifier lens to assess the formation of ulcers to measure the ulcer index. The following parameters were studied like number of ulcer, ulcer score and ulcer index along with histopathological examination. Outcomes of this work were displayed the pre-treatment of methanol root extract of C. serratum in at 200mg/kg., p.o showed significant (p≤0.001) decrease in the number of ulcer, ulcer score and ulcer index as compared to normal control animals and histopathological examination strongly support the outcomes of this dissertation. On the basis of outcome, authors concluded the C. serratum at 200mg/kg., p.o showed significant (p≤0.001) ulcer protection by minimizing the number of ulcer, ulcer score and ulcer index. This antiulcer activity of the C. serratum may be due to its antioxidant mechanism.*

***Keywords*****:** *Clerodendrum serratum; ethanolic extract; ethanol-induced ulcer; Rabeprazole*

## Introduction

An ulcer is a local defect, or excavation of the surface of an organ or tissue that is produced by shedding of inflammatory necrotic tissue. Inflammatory necrosis of mouth mucosa, stomach, intestine and lower extremities are reported in elder persons who have circulatory disturbance. Peptic ulcer disease is a group of disorders characterized by the presence of ulcer in portion of the GI tract exposed to acid in sufficient duration and concentration [1].

In Ayurveda, peptic ulcer mostly refers to *Amlapitta (amlapitta* literally means, pitta leading to sour taste.) or *Parinamasul as. Amlapitta* is a disease of the gastrointestinaltract, especially of the stomach hence, the search for an ideal anti-ulcer drug continues and has been extended to herbal drugs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse and side effects, which comes under the allopathic treatment [2].

Plants and plant-derived agents have long history as source of potential chemotherapeutic agents in Ayurveda and Unani system of medicine. *Clerodendrum serratum* (Linn), Ban-Bakri is commonly known as Bharangi in Hindi and Bhargavi in Sanskrit. It is found from 1000 to 1800 meters above mean sea level. It is distributed in warmer regions of Deccan and Carnatic, West Coast districts of Tamil Nadu, Kumaon, Sikkim, and Assam.*Clerodendrum serratum* is one of the important plants from traditional system of medicine found all over the world. They are lianas, and small trees, usually growing to 1-12 m tall, with opposite or whorled leaves [3].

*Clerodendrum serratum* is cultivated on July 2006 in Pratapgarh forest Chittorgarh district Rajasthan Rajasthan during biodiversity study. *Clerodendrum serratum* was growing wild in the ravinea of Sivaa River near Sanoti village. Locally this species is called furedetu. *Clerodendrum serratum* is a common shrub and grows along with *Lantana caora*, *Acacia nilotica* [4].

Glucose and D-mannitol, Oleanolic acid, Queretaroic acid and Serratagenic acid are present in root bark of *Clerodendrum serratum* whereas stimasterol, α-spinasterol, luteolin, luteolin-7-0 glucuronide, apigenin, baicalin and scutellarin 7-0 glucuronide are found in leaf [5].

## Material and Methods

### Collection and Authentication of Plant

Air shade dried rhizome of *Clerodendrum serratum* were collected from Jagdamba, Pharmacy, Haridwar, Uttrakhand and authenticated by Dr. H.B. Singh Scientist F and Head, Raw Materials Herbarium and Museum at National Institute of Science Communication and Information Resources, (NISCAIR) New Delhi. Reference number NISCAIR/RHMD/Consult/-2009-10/1294/97 was given to the plant sample. The rhizome was air dried in the shade at School of Pharmaceutical Sciences, Shobhit University, Meerut.

### Extraction

100 gm of air dried root powder of *Clerodendrum serratum* was placed in a Soxhlet apparatus (Perfit, India) and subjected to successive extraction using petroleum ether (40-60°C) and methanol. Subsequently, the extracts were filtered. The filtrate was evaporated using rotatory vacuum evaporator (Perfit, India).The extracts obtained after evaporation were stored in a desiccators. Petrolium ether 60-80 $^0$C temperature was used to remove fatty substances.

### Phytochemical test

Phytochemical screening of 50% methanolic extract rhizome of *Clerodendrum serratum* was performed [6].

### Antioxidant Activity

The lipid peroxides (LPOs) of stomach mucosa were determined indirectly by thiobarbituric acid reactive substances (TBARS) formation. Reduced glutathione concentration was read off a standard curve and expressed as μg GSH/g of wet tissue. Alkaline phosphatase activity was assayed using the method.

### Animals

Wister rats weighing 150-240gm of either sex were obtained from Indian Veterinary Research Institute (IVRI), Bareilly. They were kept in the departmental animal house at 26 ± 2°C and relative humidity 30-35% in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (procured from Ashirwad industries, Ropar) and water *adlibitum*. The experimental protocol to explore antiulcer potential of *Clerodendrum serratum* was approved by the Institutional Animal Ethical Committee (IAEC) 1279/ac/09/CPCSEA.

## Pharmacological screening techniques for evaluation of Antiulcer Activity

### Ethanol-Induced Acute Gastric Ulcers

Animals were fasted for 36 hours before the study but had free acess to water. Animals were randomlydivided into groups: control, standard and test. All the rats were treated orally with ethanol 90% at a dose 200gm/ml one hour after the drug treatment. The test group (extracts) 200mg/kg,p.o was administered orally. The control grsoup received only vehicle (distilled water, 10ml/kg, p.o) and standard group received Rabeprazole at a dose 20 mg/kg, p.o. After one hour, the treated animals were sacrificed with high dose of ether anesthesia. Stomach was removed from body, cut with greater curvature to measure the ulcer index [7].

The stomach were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a×5 magnifier lens to assess the formation of ulcers [8]. The numbers of ulcer were counted by using below mentioned grade as per intensity of ulcer in antrum portion of ulcer.

| | | |
|---|---|---|
| 0 | = | Normal coloured stomach |
| 0.5 | = | Red colourations |
| 1 | = | Spot ulcers |
| 2 | = | Deep ulcers |
| 3 | = | Perforated ulcers |

Ulcer index was measured by using following formula [9].

$$\mathbf{U_I = U_N + U_S + U_P \times 10^{-1}}$$

Where-

| | | |
|---|---|---|
| $U_1$ | = | Ulcer index |
| $U_N$ | = | Average number of Ulcer per animal |
| $U_S$ | = | Average number of severity score |
| $U_P$ | = | Percentage of animal with Ulcer |

Percentage inhibition of Ulceration was calculated as below:

% Inhibition of Ulceration = (Ulcer index $_{Control}$- Ulcer index $_{Test}$) ×100/ Ulcer index$_{Control}$

## Histopathology

From each group small pieces of stomach were embedded in paraffin wax. Section of 5µm thick were cut in a microtome and mounted on glass slides using standards techniques (Sairam pathology, Meerut). After staining the tissue with hematoxylin-eosin stain, the slides were viewed under a light microscope equipped for pathology [10].

## Statistical Analysis

All results were expressedas mean ±SEM and data was analyzed using one-way ANOVA followed by Post-hoc Dunnet test. $p \leq 0.05$ was considered statistically significant.

## Results

Successive extraction was carried out on the roots of *Clerodendrum serratum*. Percentage yield of successive extraction were found as 1.65 and 44.27 for petroleum ether and methanol solvent respectively.

The phytoconstituentswere identified by chemical tests, which showed the presence of various phytoconstituents such as carbohydrates, tannins, terpenoids in methanolic roots extract of *Clerodendrum serratum*.

**Table 1. Antioxidant effect of *Clerodendrum serratum* on alcohol induced ulcer model**

| Group | Lipid peroxidase(nmol MDA/g) | Reduced glutathione (µg/g) | Alkaline phosphatase(IU/I) |
|---|---|---|---|
| Group I (Control) | 38.41± 0.43 | 81.50± 2.20 | 35.22± 0.19 |
| Group II(Standard) | 26.80 ± 0.54* | 218.5± 0.74* | 21.9±0.29* |
| Group III (Treated) | 28..30± 0.46* | 203.8± 1.86* | 22.5± 0,30* |

**Effect of distilled water on ethanol induced gastric ulcer**

The distilled water (10 ml/kg, p.o), was administered one hour before administration of ethanol (1ml/200gm, p.o) in control group. The number of ulcer, ulcer score and ulcer index were found to be 6.33, 2.16 and 18 respectively shown in Figure 1, 2 and 3.

**Effect of Rabeprazole on ethanol induced gastric ulcer**

The Rabeprazole (20 mg/kg, p.o), was administered one hour before administration of ethanol (1ml/200gm, p.o) in standard group. The Rabeprazole has decreased the significantly ($p<0.01$) number of ulcer, ulcer score and ulcer index 0.416, 0.333, 5.833 respectively as compared to control group shown in Figure 1,2 and 3.

**Effect of methanolic roots extract of *Clerodendrum serratum* at a dose (200mg/kg, p.o) in ethanol induced ulcer**

Administration of roots extract of *Clerodendrum serratum* at a dose (200mg/kg/p.o) one hour before administration of ethanol (1ml/200gm, p.o) has significantly ($p<0.01$) reduced number of ulcer, ulcer score and ulcer index 1.3, 0.833, 8.66 respectively as compared to control group shown in Figure 1,2 and 3.

**Effect of methanolic roots extract of *Clerodendrum serratum* at a dose (200mg/kg, p.o)**

Ethanol administration was found to increase lipid peroxidation, alkaline phosphate and reduced glutathione in the control group when compared to normal control rats. Administration of *Clerodendrum serratum* significantly decreased lipid peroxidation and alkaline phosphate whereas the reduced glutathione were significantly increased at the dose levels of 200 mg/kg.

**Histopathological study of stomach mucosa of wistar rats using ethanol induced ulcer**

Transverse sections of stomach of control animals show hemorrhage and discontinuity in the lining epithelium hyperplastic mucosal glands. The treated animal methanolic roots extract of *Clerodendrum serratum* (200 mg/kg, p.o) show no ulcer formation, small atrophic glands, thick muscularis, and edematous sub-mucosa with inflammatory infiltrate. However, Rabeprazole has significant effect on ulcer formation, mild hyperplastic mucosa without any edema formation Figure 4.

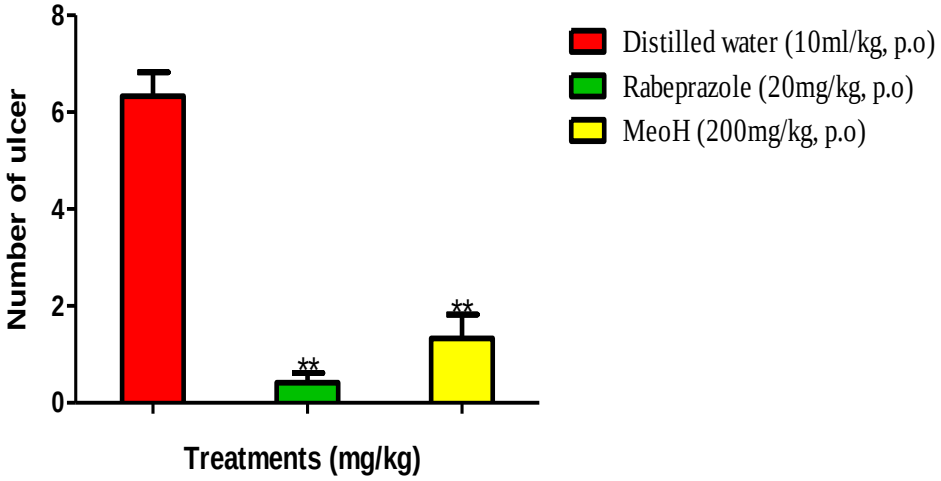



**Figure 1. Effect of methanolic root extract of *Clerodendrum serratum* on number of ulcer in ethanol induced ulcer**

The distilled water (10 ml/kg, p.o), Rabeprazole, a standard drug (20 mg/kg, p.o) and methanolic root extract of *Clerodendrum serratum* (200 mg/kg, p.o) were respectively administered one hour before administration of ethanol in control, standard and extract treated group. **p<0.01 was considered to be significantly different in comparison with control group.

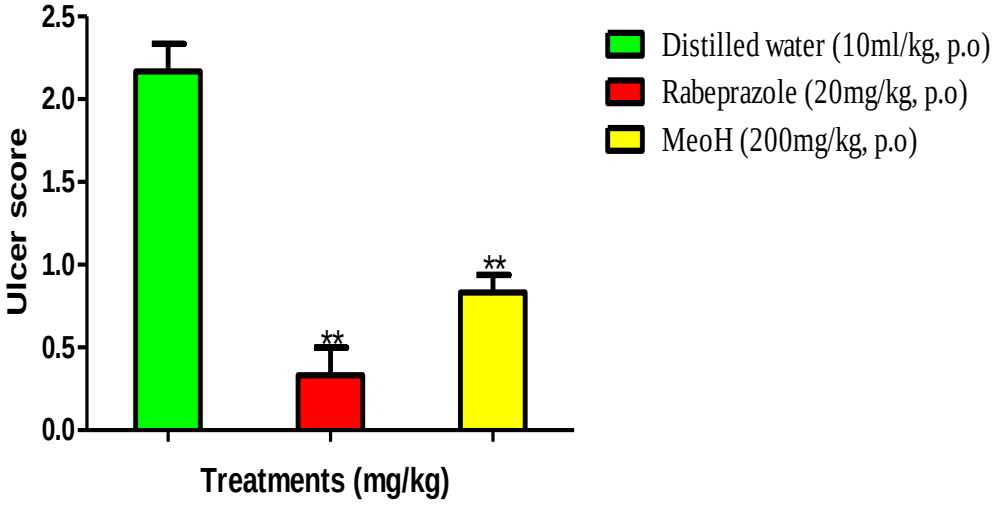


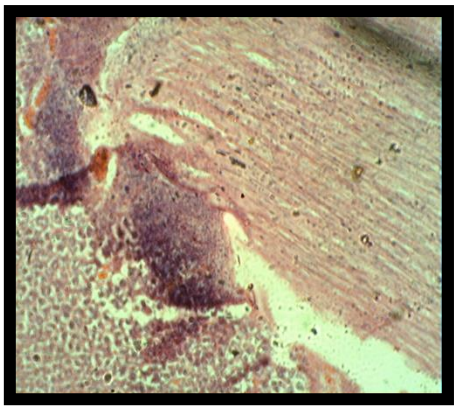

**Figure 2. Effect of methanolic root extract of *Clerodendrum serratum* on ulcer score in ethanol induced ulcer**

The distilled water (10 ml/kg, p.o), Rabeprazole, a standard drug (20 mg/kg, p.o) and methanolic root extract of *Clerodendrum serratum* (200 mg/kg. p.o) were respectively administered one hour before administration of ethanol in control, standard and extract treated group. **p<0.01 was considered to be significantly different in comparison with control group.

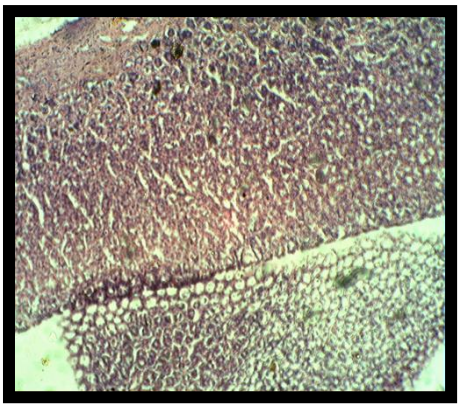
**Ulcers**

**Figure 3. Effect methanolic root extract of *Clerodendrum serratum* on ulcer index in ethanol-induced Ulcers**

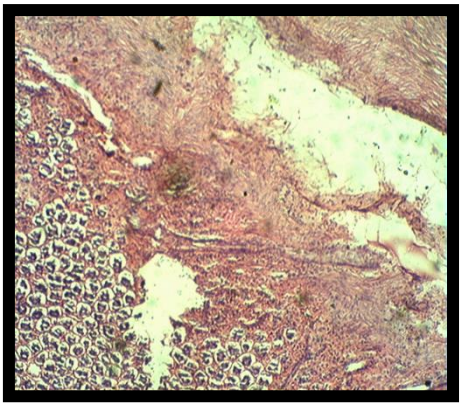
The distilled water at a dose (10 ml/kg, p.o), Rabeprazole, a standard drug (20mg/kg, p.o) and methanolic root extract of *Clerodendrum serratum* (200mg/kg, p.o) were respectively administered one hour before administration of ethanol in control, standard and extract treated group. **p<0.01 was considered significantly different in comparison with control group



**(a)**



**(b)**



**(c)**

**Figure 4. Histopathological section of stomach mucosa in wistar rat using ethanol induced ulcers (a, b and c)**

**(a**) Transverse section of stomach mucosa of animals treated with distilled water 10 ml/kg, p.o
**(b**) Transverse section of stomach mucosa of animals treated with Rabeprazole standard drug (20 mg/kg, p.o)– No ulcer formation, mild hyperplastic mucosa without any edema formation.
**(c**) Transverse section of stomach mucosa of animals treated with methanolic roots extract of *Clerodendrum serratum* 200 mg/kg, p.o – No ulcer formation, small atrophic glands, thick muscularis and edematous sub-mucosa with inflammatory infiltrate.

## Discussion

In ethanol induced model methanolic root extract of *Clerodendrum serratum* at a dose of (200 mg/kg, p.o) has produced significant inhibition of ulcerative lesion by 53.2% as compared to the control group. Rabeprazole significantly decreased number of ulcer, ulcer score and ulcer index as compared to the control value.[11]. Alcohol induced ulcers are not inhibited by antisecretory agents such as cimetidine, but are inhibited by agents that enhance mucosal defensive factors such as prostaglandins.

The antiulcer activity exhibited by the extract are probably responsible for the synthesis of mucus, phospholipid, bicarbonate and prostaglandins as well as reduced acid and pepsin outputs, consequently promoting the inhibition of gastric-acid secretion [12].

These results of *C. serratum* extracts probably have dose dependent an antiulcerogenic effect that may be related to cytoprotective activity, as the extract presented significant results in the ethanol induced ulcer. Ethanol produced reactive species, which are responsible for mucosal injury and lipid peroxidation, a free radicle mediated process that ultimately destroys lipid membrane. Such injuries are often associated with extensive lesions of mucosal capillaries increased vascular permeability and reduction of mucosal blood flow [13].

The phytoconstituents were identified by chemical tests, which showed the presence of various phytoconstituents (carbohydrate, tannin, terpenoid) in methanolic roots extract of *Clerodendrum serratum*. Amongst these secondary compounds, triterpenoids are referred as antiulcer activity [14]. Tannins are one of most important botanical compounds with anti-ulcer and gastroprotective activities. Antioxidant effects of methanolic extract roots of *Clerodendrum serratum* (CSR) at various concentrations in the DPPH radical scavenging assay FRAP assay (ferric reducing antioxidant power) and the hydrogen peroxide radical scavenging assay. Antioxidants are known to inhibit lipid peroxidation and scavenge free radicals. The protection from ulcer produced by the extract likely suggests the ability of extracts to enhance cytoprotective mechanism and inhibit reactive species mediated lesion in mucosa. Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids. Biological membranes are often rich in unsaturated fatty acids. Therefore, it is not surprising that membrane lipids are susceptible

to peroxidative attack. Similarly, present study showed that there was a significant increase in lipid-peroxidation in rat stomach tissues of control animals. However, significant decrease in lipid-peroxidation was observed by the administration of 200 mg/kg of *C.serratum* in experimental models [15].

GSH is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation [16]. Administration of methanolic extract of roots of C.*serratum* resulted in a significant increase in the GSH levels as compared to the control animals, which suggests its efficacy in preventing free radical induced damage [17].

Antiulcer activity of methenolic root extract of *Clerodendrum serratum* is attributed cytoprotective and antioxidant mechanism and presence of tannins and triterpenoids, which may improve. The protection from ulcer produced by the extract likely suggests the ability of extracts to enhance cytoprotective mechanism and inhibhit reactive species mediated lesion in mucosa. Antiulcer activity of methenolic root extract of *Clerodendrum serratum* is attributed cytoprotective and antioxidant mechanism and presence of tannins and triterpenoids, which may improve.

## Conclusion

This study showed that the 50% methanolic extract of *Clerodendrum serratum* roots has antiulcer effects that were proven by biochemical and histopathological analysis. The methanol extract of *Clerodendrum serratum* roots possess significant antiulcer property in a dose dependent manner by improving gastric mucosal defence mechanism.

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