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EVALUATION OF ANTI ULCER AND *IN-VITRO* ANTIOXIDANT ACTIVITIES OF AQUEOUS AND METHANOLIC EXTRACTS OF *NEOLAMARCKIA CADAMBA* LEAVES AND BARK IN WISTAR ALBINO RATS

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Keywords:

Antiulcer activity, *Neolamarckia cadamba*, *in vitro* antioxidant, pyloric ligation, Aspirin induced, DPPH

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ABSTRACT: The antiulcer activity of Aqueous and Methanolic extracts of *Neolamarckia cadamba* leaves and bark was investigated in Pylorus ligation and Aspirin induced ulcer models in Wistar albino rats. In both models the common parameter determined was ulcer index. Aqueous extract of *Neolamarckia cadamba* leaves (AENCL) at dose of 200mg/kg and 400 mg/kg produced significant inhibition of gastric lesion induced by Pylorus ligation induced ulcer and Aspirin induced gastric ulcer. The AENCL (200mg/kg and 400mg/kg) showed significant ($p < 0.05$) reduction in gastric volume, pH, free acidity, Total acidity, ulcer index and % ulcer inhibition was compared to control. This present study indicates that AENCL and Methanolic extract of *Neolamarckia cadamba* leaf (MENCL) have potential antiulcer activity in both models. These results may further suggest that the AENCL and MENCL possess antiulcerogenic as well as ulcer healing properties which might be due to its antisecretory activity. Antioxidant activity was evaluated for free radical scavenging activity by DPPH assay which showed the significant antioxidant activity of AENCL and MENCL further supported their antiulcerogenic property.

INTRODUCTION: The peptic ulcer describes a condition in which there is a discontinuity in the entire thickness of gastric or duodenal mucosa that persists as a result of acid and pepsin in gastric juice¹. Ulcers are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach². Herbal medicine is fast emerging treatment as an alternative to available synthetic drugs for treatment of ulcer, possibly due to lower costs and less side effects.

Various chemical compounds have been isolated from medicinal plants with antiulcer activity^{3, 4}. Leaves of *Neolamarckia cadamba* (Rubiaceae) are commonly used as vegetable throughout India especially in southern parts.

Apart from their use as wound healing, leaf and bark of the plant are reported to possess medicinal values such as anti-inflammatory, hypolipidemic, analgesic, antimicrobial, anthelmintic and antidiabetic activities⁵⁻⁷. It is a remedy for fever, inflammation of eyes, snake-bite. The present study assesses the effect of aqueous and methanolic extracts of *Neolamarckia cadamba* leaves and bark on pyloric ligation and aspirin induced gastric ulcers in rats to determine its effect on gastric secretion, development of gastric ulcers in rats and also involves *in-vitro* antioxidant activity.

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MATERIALS AND METHODS:

Plant material: The fresh leaves and bark of *Neolamarckia cadamba* were collected from Bhimavaram, West Godavari district, Andhra Pradesh. The plant material was identified and authenticated by Mrs. P. Prasanna kumari, Head of the Department of Botany, D.N.R (A) College, Bhimavaram. These fresh leaves and bark were washed thoroughly with tap water followed by distilled water to remove the earthy matters and freed from debris.

Preparation of extracts: Aqueous extracts: The leaf and bark powders were macerated in water for 4 days by adding few ml of chloroform as preservative. Later it was filtered and the filtrate was collected and air dried, by putting under the fan. Then the dried extract was collected and stored in desiccator for further use.

Methanolic extracts: The leaf and bark powders were successively extracted using soxhlet extractor with methanol. The extract obtained was concentrated by using Rota evaporator. The concentrated extract was dried and stored in desiccator for further use.

Preliminary phytochemical studies: Aqueous extract of *Neolamarckia cadamba* leaves (AENCL), Aqueous extract of *Neolamarckia cadamba* bark (AENCB), Methanolic extract of *Neolamarckia cadamba* leaves (MENCL), Methanolic extract of *Neolamarckia cadamba* bark (MENCB) were subjected to preliminary phytochemical screening for the detection of various plant constituents.

Animals: Wistar albino rats of either sex weighing between 150-200g were procured from animal house, Shri Vishnu College of Pharmacy, Bhimavaram. The animals were housed under standard laboratory conditions of temperature (25 ± 2 °C) and relative humidity ($55\pm 5\%$) with a 12:12 light-dark cycle in polypropylene cages.

All procedures involving animals were conducted as per the norms of Institutional Animal Ethics Committee (IAEC) approval (439/PO/01/a/CPCSEA).

Acute oral toxicity studies: Toxicity studies of all the four extracts were carried out in Swiss albino mice weighing between 20-25g. They were performed according to OECD guideline No. 423. Four groups of mice comprising six animals each were treated with 100, 200, 400, 800, 1000 and 2000mg/kg of the extracts were suspended in 0.5% w/v SCMC were administered orally, via gastric catheter. The animals were then observed continuously for the first 4hrs for any behavioural changes and for mortality if any at the end of 72hrs. All four extracts were found to be safe since no animal died even at the dose of 2000 mg/kg when administered orally and the animals did not showed any gross behavioural changes.

Assessment of Antiulcer activity:

Pyloric ligation induced ulcer: Animals were divided into ten groups of six animals each. Group I served as control and received distilled water and group II was served as standard and administered with Omeprazole 20mg/kg p.o. Group III and group IV was treated with Aqueous Extract of *Neolamarckia cadamba* leaves 200mg/kg and 400mg/kg p.o. respectively. Group V and group VI were treated with Methanolic Extract of *Neolamarckia cadamba* leaves 200mg/kg and 400mg/kg p.o respectively. Group VII and group VIII were treated with Aqueous Extract of *Neolamarckia cadamba* bark 200mg/kg and 400mg/kg p.o respectively. Group IX and group X were treated with Methanolic extract of *Neolamarckia cadamba* bark 200mg/kg and 400mg/kg p.o respectively.

The drugs were administered daily for 5 days. On the 5th day, the rats were fasted for overnight before pyloric ligation with free access to drinking water in individual cages with raised bottoms of wide wire mesh to avoid cannibalism and coprophagy⁸. At the end of 24hrs, the rats were anaesthetized with ketamine 80 mg/kg. Abdomen was opened by a midline incision of two inches. The stomach was carefully lifted out and a ligature was placed at the pyloric sphincter without causing any damage to its blood supply and traction on the pylorus. The stomach was replaced carefully and the abdominal wall was closed with interrupted sutures.

The animals were deprived of food and water post operatively and were sacrificed after 6 hours of pyloric ligation. The oesophagus connecting the stomach was ligated to prevent the escape of gastric contents. The stomach was isolated and the gastric contents were collected. Volume of the gastric secretion and pH of the gastric juice were determined along with free acidity and total acidity⁹.

Macroscopic evaluation of stomach: The stomachs were opened along the greater curvature, washed gently with saline water to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. The scoring of ulcers was done by the method suggested by Kulkarni S.K et al¹⁰. Scoring of ulcer was made as follows: 0 = normal coloured stomach, 0.5 = red coloration, 1 = spot ulcers, 1.5 = haemorrhagic streaks, 2 = ulcers $\geq 3 \leq 5$, 3 = ulcers ≥ 5 .

Estimation of Ulcer index: Mean ulcer score for each animal is expressed as ulcer index.

Estimation of percentage of ulcer inhibition: The percentage of ulcer inhibition is obtained by following formula;

$$\frac{(\text{Control mean UI} - \text{Test mean UI})}{\text{Control mean UI}} \times 100$$

Estimation of gastric volume: The gastric juice collected from each stomach was drained into test tubes and then centrifuged at 1000 rpm for 10 min and the volume of supernatant was noted¹¹.

Estimation of pH of gastric juice: The pH of the gastric juice was recorded by pH meter.

Estimation of Free acidity and Total acidity: 1 ml of gastric juice was pipetted into 100 ml conical flask. It was diluted to 10 ml with distilled water and added 2-3 drops of Topfer's reagent. It was titrated with 0.01 N sodium hydroxide until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of the alkali added was noted. This volume corresponds to free acidity. Then, 2-3 drops of phenolphthalein solution was added and titration

was continued until pink colour reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity.

Acidity can be calculated by using the formula:

$$\text{Acidity} = \frac{\text{Vol.of NaOH} \times \text{Normality} \times 100}{0.1} \text{ mEq/L/100 gm}$$

Aspirin induced ulcer model: The ulcer was administered by using aspirin. Animals were divided into ten groups of six animals each and were treated with respective drugs for 5 days as mentioned in previous model. On the 5th day, the rats were fasted for 24hrs with free access to drinking water in individual cages with raised bottoms of wide wire mesh to avoid cannibalism and coprophagy. At the end of 24hrs, aspirin (400mg/kg) with DMSO was administered to the rats of all the groups to induce the ulcer.

After 6hrs rats were sacrificed and stomachs were dissected out and the gastric contents were collected. The stomachs were opened along the greater curvature and were examined to determine the ulcer index and percentage of ulcer inhibition. From the collected gastric contents, volume of gastric volume, pH of gastric juice, free acidity and total acidity were estimated similar to pylorus ligation induced ulcer model¹².

Assessment of *in-vitro* antioxidant activity:

Determination of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) Radical Scavenging Activity: An aliquot of 3ml of 0.04% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30min. decolourization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared by using 0.1ml of respective vehicle in the place of ascorbic acid/plant extract.

Calculation of percentage inhibition: The percentage inhibition of lipid peroxidation by the extract was calculated using the formula:

$$\text{Inhibitory ratio} = (A_0 - A_1) \times 100 / A_0$$

Where A_0 is the absorbance of control and A_1 is the absorbance of the test sample.

Statistical analysis: Mean values \pm S.E.M were calculated for each parameter. For the determination of significant difference between the

groups, each parameter was analysed separately and one-way analysis of variance (ANOVA) was carried out followed by Dunnet's test. The difference was considered significant if $P < 0.05$.

RESULTS:

TABLE 1: EFFECT OF AQUEOUS AND METHANOLIC EXTRACT OF NEOLAMARCKIA CADAMBA LEAVES AND BARK ON GASTRIC VOLUME, PH, FREE ACIDITY, TOTAL ACIDITY, MEAN ULCER INDEX AND % OF ULCER INHIBITION IN PYLORIC LIGATION INDUCED ULCER MODEL

| Group | Treatment | Dose mg/kg | Gastric volume (ml) | pH | Free acidity (mEq/L) | Total acidity (mEq/L) | Mean ulcer index | % of ulcer inhibition |
|-------|----------------------------|------------|---------------------|-------------------|----------------------|-----------------------|-------------------|-----------------------|
| I | Solvent-Control | 10ml/kg | 9.25 \pm 0.25 | 2.75 \pm 0.50 | 107 \pm 3.0 | 115.5 \pm 0.50 | 5.83 \pm 0.08 | - |
| II | Standard drug - Omeprazole | 20mg/kg | 5.2 \pm 0.55*** | 4.2 \pm 0.65** | 31 \pm 2.5*** | 39 \pm 3.2*** | 1.0 \pm 0.04*** | 82.84 |
| III | AENCL | 200mg/kg | 6.5 \pm 0.50*** | 4.25 \pm 0.25** | 39 \pm 1.00*** | 45 \pm 3.00*** | 3.0 \pm 0.02*** | 48.54 |
| IV | AENCL | 400mg/kg | 1.4 \pm 0.60*** | 4.75 \pm 0.25** | 26.5 \pm 1.50*** | 35.5 \pm 0.50*** | 2.0 \pm 0.41*** | 65.69 |
| V | MENCL | 200mg/kg | 7.75 \pm 0.25 | 3.55 \pm 0.25 | 46.5 \pm 1.50*** | 54 \pm 2.00*** | 4.0 \pm 0.62** | 31.38 |
| IV | MENCL | 400mg/kg | 1.75 \pm 0.05 | 4.5 \pm 0.42* | 41 \pm 1.02*** | 46.5 \pm 1.50*** | 3.50 \pm 0.3*** | 39.96 |
| VII | AENCB | 200mg/kg | 6.28 \pm 0.21*** | 3 \pm 0.28 | 46.3 \pm 0.10*** | 56.2 \pm 0.16*** | 3.75 \pm 0.68** | 35.67 |
| VIII | AENCB | 400mg/kg | 6.26 \pm 0.18*** | 4.41 \pm 0.23* | 35 \pm 0.09*** | 41.6 \pm 0.08*** | 3.25 \pm 0.91** | 44.25 |
| IX | MENCB | 200mg/kg | 7.15 \pm 0.12*** | 4.1 \pm 0.38* | 47.6 \pm 0.08*** | 55.5 \pm 0.11*** | 4.5 \pm 0.8** | 22.81 |
| X | MENCB | 400mg/kg | 6.78 \pm 0.11*** | 4.23 \pm 0.25* | 42.8 \pm 0.14*** | 51.5 \pm 0.10*** | 4.0 \pm 0.52** | 31.38 |

a. Values are expressed as Mean \pm S.E.M. b. One way ANOVA followed by Dunnet's t - test. c. *** $p < 0.01$, ** $p < 0.05$, * $p < 0.1$ as compared to control group

TABLE 2: EFFECT OF AQUEOUS AND METHANOLIC EXTRACT OF NEOLAMARCKIA CADAMBA LEAVES AND BARK ON GASTRIC VOLUME, PH, FREE ACIDITY, TOTAL ACIDITY, MEAN ULCER INDEX AND % OF ULCER INHIBITION IN ASPIRIN INDUCED ULCER MODEL

| Group | Treatment | Dose mg/kg | Gastric volume (ml) | pH | Free acidity (mEq/L) | Total acidity (mEq/L) | Mean ulcer index | % of ulcer inhibition |
|-------|----------------------------|------------|---------------------|--------------------|----------------------|-----------------------|--------------------|-----------------------|
| I | Aspirin-Control | 400ml/kg | 1.5 \pm 0.001 | 2.5 \pm 0.18 | 116 \pm 0.22 | 120 \pm 0.18 | 6.33 \pm 0.11 | - |
| II | Standard drug - Omeprazole | 20mg/kg | 1 \pm 0.11 | 4.46 \pm 0.16*** | 30.5 \pm 0.12*** | 42 \pm 0.20*** | 1.33 \pm 0.1*** | 78.98 |
| III | AENCL | 200mg/kg | 1.3 \pm 0.22 | 3.4 \pm 0.16** | 31.6 \pm 0.2*** | 37.2 \pm 0.65*** | 2.5 \pm 0.90*** | 60.50 |
| IV | AENCL | 400mg/kg | 0.9 \pm 0.20** | 4.53 \pm 0.15*** | 30.9 \pm 0.4*** | 36.8 \pm 0.8*** | 2.0 \pm 0.18*** | 68.4 |
| V | MENCL | 200mg/kg | 1.5 \pm 0.10 | 2.85 \pm 0.10 | 44.6 \pm 0.6*** | 50.1 \pm 0.72*** | 3.5 \pm 0.65*** | 44.70 |
| IV | MENCL | 400mg/kg | 1.4 \pm 0.11 | 3.46 \pm 0.13** | 38.4 \pm 1.1*** | 47.8 \pm 0.67*** | 2.5 \pm 0.21*** | 0.50 \pm 1.04 |
| VII | AENCB | 200mg/kg | 1.2 \pm 0.24 | 3.1 \pm 0.12 | 44.8 \pm 0.61*** | 50.4 \pm 0.42*** | 3.5 \pm 0.72*** | 44.70 \pm 0.48 |
| VIII | AENCB | 400mg/kg | 1.1 \pm 0.02 | 3.33 \pm 0.09** | 32.9 \pm 0.8*** | 38.6 \pm 0.83*** | 3.0 \pm 0.8*** | 52.33 \pm 0.68 |
| IX | MENCB | 200mg/kg | 1.4 \pm 0.11 | 3.41 \pm 0.24** | 44.5 \pm 1.2*** | 49.8 \pm 0.80*** | 4.0 \pm 0.72*** | 36.80 \pm 0.25 |
| X | MENCB | 400mg/kg | 1.3 \pm 0.01 | 3.9 \pm 0.08*** | 44.2 \pm 0.8*** | 49.2 \pm 0.68*** | 3.75 \pm 0.65*** | 40.75 \pm 0.76 |

a. Values are expressed as Mean \pm S.E.M. b. One way ANOVA followed by Dunnet's t - test. c. *** $p < 0.01$, ** $p < 0.05$, * $p < 0.1$ as compared to control group

TABLE 3: PHYTOCHEMICAL CONSTITUENTS OF AQUEOUS AND METHANOLIC EXTRACTS OF NEOLAMARCKIA CADAMBA LEAVES AND BARK

| S. No. | Phyto constituents | AENCL | MENCL | AENCB | AENCB |
|--------|---------------------|-------|-------|-------|-------|
| 1 | Alkaloids | + | + | + | + |
| 2 | Flavonoids | + | - | + | + |
| 3 | Glycosides | + | + | + | + |
| 4 | Phenols | + | + | + | + |
| 5 | Saponins | + | - | + | + |
| 6 | Carbohydrates | + | + | + | + |
| 7 | Tannins | + | + | + | + |
| 8 | Terpenoids | + | - | - | - |
| 9 | Fixed oils and fats | - | - | - | - |

TABLE 4: IN-VITRO ANTIOXIDANT ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF NEOLAMARCKIA CADAMBA LEAVES AND BARK

| Concentration | Ascorbic acid | AENCL | MENCL | AENCB | MENCB |
|---------------|-----------------|------------------|------------------|------------------|------------------|
| 5 μ | 48.1 \pm 0.26 | 48.81 \pm 0.52 | 30.73 \pm 0.44 | 24.32 \pm 0.56 | 31.43 \pm 0.51 |
| 10 μ | 55.2 \pm 0.75 | 56.31 \pm 0.30 | 33.11 \pm 0.21 | 32.53 \pm 0.91 | 49.82 \pm 0.34 |
| 25 μ | 56.6 \pm 0.66 | 76.71 \pm 0.26 | 43.79 \pm 0.34 | 51.45 \pm 0.54 | 67.17 \pm 0.22 |
| 50 μ | 64.8 \pm 2.08 | 80.83 \pm 0.72 | 51.76 \pm 0.04 | 68.53 \pm 0.36 | 70.94 \pm 0.16 |
| 100 μ | 84.9 \pm 0.53 | 82.76 \pm 0.46 | 80.47 \pm 0.23 | 74.32 \pm 0.28 | 72.57 \pm 0.12 |

Macroscopic appearance of the gastric mucosa in pyloric ligation induced ulcer model:



FIG. 1: GROUP I (CONTROL)



FIG. 2: GROUP II (STANDARD)



FIG. 3: GROUP IV (AENCL 400MG/KG)

Macroscopic appearance of the gastric mucosa in Aspirin induced ulcer model:



FIG. 4: GROUP I (CONTROL)



FIG-5: GROUP II (STANDARD)



FIG-6: GROUP IV (AENCL 400MG/KG)

HISTOPATHOLOGY:

Histopathological studies showing effect of Aqueous extract of *Neolamarckia cadamba* leaf in pyloric ligation induced ulcer model:

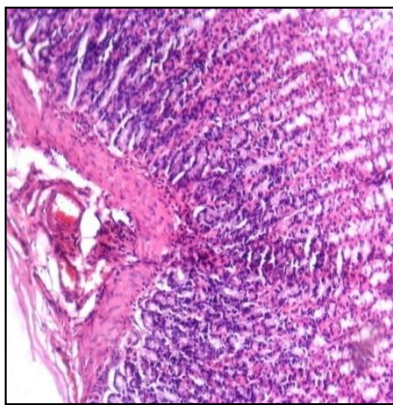


FIG. 7:
(A- CONTROL)

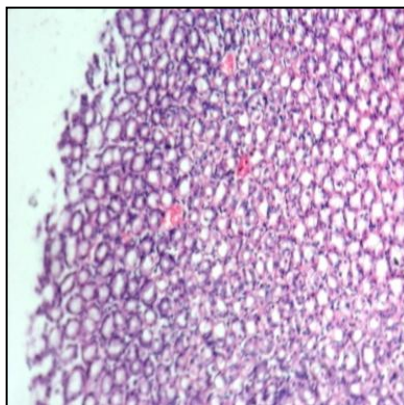


FIG. 8:
(B-STANDARD)
(OMEPRAZOLE)



FIG. 9:
C-AENCL (400MG/KG)

A) Control: Rat stomach showing severe ulcer lesions and desquamation of the surface epithelium in pyloric ligation gastric ulcers. **B)** Standard: Rat stomach fairly protected with Omeprazole (20mg/kg) in pyloric ligation induced ulceration. **C)** AENCL (400mg/kg): Rat stomach showing a protected epithelium due to Aqueous extracts of *Neolamarckia cadamba* leaves (400mg/kg) in pyloric ligation induced gastric ulceration.

Histopathology studies showing effect of Aqueous extract of *Neolamarckia cadamba* leaf in Aspirin induced Ulcer model:

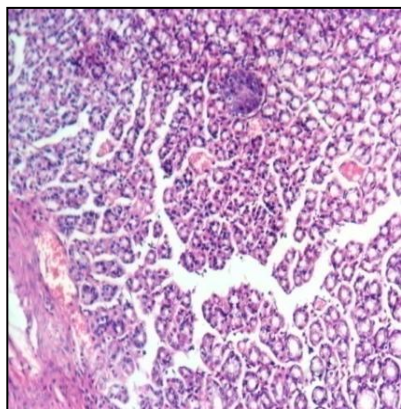


FIG. 12:
(A- CONTROL)

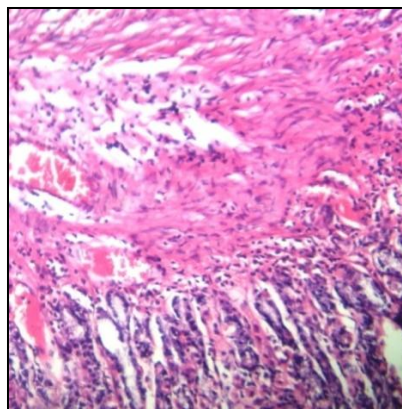


FIG. 13:
(B- STANDARD)
(OMEPRAZOLE)

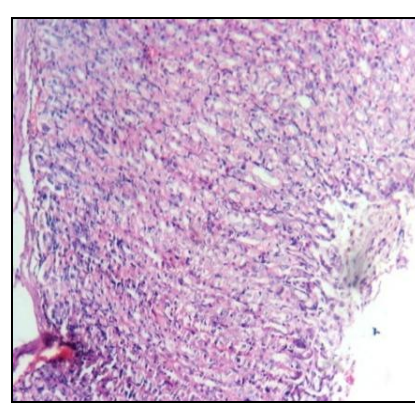


FIG. 14:
C- AENCL (400MG/KG)

A) Stomach of control rat showing erosion in the upper part of epithelium with RBCs in eroded portion. **B)** Stomach of rat treated with Omeprazole showing small erosions with minimum deviation from normal morphology. **C)** Stomach of rat treated with AENCL (400mg/kg) showing superficial erosions with minimum deviations from normal morphology.

DISCUSSION: It is evident from the result of the present investigation that the aqueous extract of *Neolamarckia cadamba* leaves possesses antiulcer activity in aspirin induced and Pylorus ligation induced ulcer model. Plant leaf and bark aqueous extracts provided more consistent antiulcer activity compared to those of methanolic extracts. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse. Water was used as extraction solvent due to better extraction efficiency, shorter extraction time, and elimination of the need to boil or homogenize the samples. From the references it is observed aqueous extract of *Neolamarckia cadamba* leaves containing glycosides, tannins, flavonoids and saponins having antiseptic activity. Further, Aqueous extract of *Neolamarckia cadamba* leaf contain terpenoids in addition which may be responsible for more effect on ulcer inhibition¹³.

The antiulcer activity of the aqueous extract of *Neolamarckia cadamba* leaf can be compared to the activity of the standard drug Omeprazole. When the results are compared, *Neolamarckia cadamba* Leaf extracts showed better activity than bark extracts in Pylorus ligation induced ulcers and Aspirin induced ulcers. The results of the present study suggest that the aqueous extract of *Neolamarckia cadamba* leaf may be beneficial in the treatment of gastric lesions.

CONCLUSION: It can be summarized that the aqueous extract of *Neolamarckia cadamba* leaf possess the antiulcer activity against the Pyloric ligation and Aspirin induced gastric ulceration animal model in rats. Among the two doses (200mg/kg and 400mg/kg), 400mg/kg AENCL produced significant antiulcer and antioxidant activity. AENCL produced significant antiulcer activity with that of standard drug omeprazole. AENCL and MENCL showed significant antioxidant activity with that of standard drug Ascorbic acid. The AENCL showed better activity

compared to MENCL, AENCB and MENCB. Plant extracts showed better activity in Pyloric ligation induced ulcer model than in Aspirin induced Ulcer model. Further investigation is to be carried out to isolate the active compounds and to elucidate the exact mechanism of antiulcer activity through the use of additional experimental models¹⁴.

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