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Research Article

# A Pharmacological Study of Gastric Antiulcer Activity of the Leaf Extracts of *Murraya koenigii*

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## Abstract

*Murraya koenigii* Linn (MKL) is a native plant of India, Sri Lanka and other countries from South Asia. It has been used by the people of this region as a traditional cure in various ailments including gastric ulcer. Antiulcer activity of aqueous extracts of fresh leaves of MKL at the doses of 200 mg and 400 mg per kg were tested among different groups of Wistar rats of either sex in the experimental models of ulcer induction by pyloric ligation and by cold and restraint stress and compared with a standard drug ranitidine. The present study demonstrated the antiulcer activity of aqueous extract of leaves of MKL in animal models of pyloric ligation and cold restraint induced gastric ulcers. The acute toxicity study by the administration of doses of MKL upto 2000 mg/kg in the above for a period of 14 days did not show any serious toxicity. The available evidence of antiulcer activity provides satisfactory evidence further scientific studies using advanced methods for testing its usefulness in human. The ulcer protection in ranitidine group was 64.12%; in groups treated MKL in doses of 200 and 400 mg/kg, it was 45.2% and 50.07%, respectively.

**Keywords:** *Murraya koenigii* Linn.; Gastric Ulcer; Antiulcer Activity

## Introduction

*Murraya koenigii* Linn (MKL) is a native plant of India, Sri Lanka and other countries from South Asia. The plant is distributed all over India with abundance in Sikkim, Garhwal, West Bengal, Assam, Western ghats and Kerala [1]. It grows as a small tree or deciduous shrub with a height of 6 meters. The trunk is short, 15 to 40 cm in diameter, smooth, brown or grey bark and a dense shady crown [2]. The leaf margins have irregular serrations and petiole is 2 to 3 mm long. It belongs to Class Magnoliopsida, subclass Rosidae and family Rutaceae. It is known by various vernacular names in different languages, but the most commonly as Curry leaf tree. On phytochemical investigations, it was found to contain alkaloids, volatile oil gycozoline, xanthotoxine and sesquiterpione. The leaf has been found to show antioxidant activity, hypoglycemic activity, antibacterial activity6, anti-dysentery8, hepatoprotective and also have antiulcer activity [1,4-6].

## Method and Materials

The present study was conducted in the department of pharmacology of Gauhati Medical College & Hospital, Guwahati after obtaining approval from the Animal Ethics Committee of Gauhati Medical College and Hospital, Guwahati with CPCSEA Registration No. 351: 03/01/2001 and study protocol approval No. MC/05/2015/88).

The experiment was conducted in 54 Wistar rats of either sex in the weight range of 150-250 grams. All the animals were housed in the Animal House of our Institute, in a clean area. The tempera-

ture was controlled at 24 ± 2° C, with relative humidity of 30-70%, with a light and dark cycle of 12 hours each. Six rats per cage were housed in polypropylene cages during the study. Cages had a stainless-steel top grill having facilities for food and drinking water in polypropylene bottles with stainless steel sipper tube. Standard rat pellet feed and pure drinking water was provided *ad libitum*. Maintenance of the study animals were done in strict accordance with the CPCSEA guidelines.

## Materials

Drugs and chemicals: (1) Ranitidine (2) Aqueous extract of *Murraya koenigii* (3) Normal Saline (0.9% NaCl) (4) Topfer Reagent (5) 0.1 N NaOH solution (6) Phenolphthalein solution

## Plant material

Fresh leaves of MKL were obtained from the market. Specimens were collected in the month of September, 2016. Authentication and verification of the plants were done by proper authorities.

## Preparation of aqueous extract

The leaves were thoroughly washed and shade dried, grinded into fine powders and stored in air tight containers. The soxhlet apparatus was used for extraction. The resultant extracts were filtered using Whatman filter paper no. 1, concentrated by evaporation and collected in petri dishes. The final yield of MKL leaf extracts were 66.3 grams (26.5%), stored in a refrigerator at 4 °C in labelled air tight containers.

## Acute toxicity tests [6]:

This was carried out as per OECD guidelines. The rats were randomly selected, marked for identification, and kept in their cages for seven days prior to dosing. Animals were fasted prior to dosing (food was withheld for 3-4 hours). The dose was calculated according to the fasted body weight. Post fasting, the first animal was dosed at 175 mg/kg body weight with aqueous extract of study drug by gavage. Food was withheld for a further 1-2 hours. The animal was observed for mortality for 48 hours. Then a second animal was dosed at 550 mg/kg body weight by the same process. Again after 48 hours a third animal was dosed at 2000 mg/kg and observed for the next 48 hours for mortality. All the above animals were observed for a period of 14 days and were found to be alive at 2000 mg/kg. Two doses of the study drug were selected i.e. 200 mg/kg, and 400 mg/kg.

## Study groups:

The study had 2 experimental models.

- Ulcer induction by pyloric ligation [7]
- Ulcer induction by cold and restraint stress [8]

For each model, 5 groups of 6 animals each were selected. Group I (Normal control) served as a common for both models. Thus, for both models, a total of 54 animals were taken, detailed as follows [9-11].

| Groups | Group code | Treatment given |
|---|---|---|
| Normal Control | NC | No induction or intervention given |
| Disease Control | DC | Normal saline 1 ml/kg |
| Standard | R20 | Ranitidine 20 mg/kg |
| *Murraya koenigii* aqueous extract low dose | AEMK200 | *Murraya koenigii* aqueous extract 200 mg/kg |
| *Murraya koenigii* aqueous extract high dose | AEMK400 | *Murraya koenigii* aqueous extract 400 mg/kg |

**Table 1:** Duration and route of drug administration: All study drugs were administered orally for 7 days, using an oral feeding tube for rats.

## Study procedure

### Ulcer induction by pylorus ligation by Shay's method

Under proper aseptic and antiseptic conditions, ulcer induction was done by pylorus ligation as per the standard procedure [7].

### Ulcer induction by cold and restraint stress by Vincent., *et al.*:

Under proper aseptic and antiseptic conditions, the animals were starved and on 7th day, ulcers were induced by cold while putting the animal in restraint [8].

## Variables assessed in the study [12]:

Following variables were measured in both models in the study:

- Ulcer index
- Percentage of ulcer protection
- Additionally following variables were studied in pyloric ligation model,

- Gastric content volume
- Free acidity
- Total acidity
- pH

### Estimation of variables

The ulcers were evaluated quantitatively using ulcer index and scoring number [13]. Percentage ulcer protection was also calculated. Volume, pH, free and total acidities of gastric content was determined. Titration was done with 0.01 N NaOH, till total acidity was achieved.

### Histopathological examination

Stomach tissues were fixed in 10% formalin for 24 hrs, then embedded in paraffin. Small sections were made (3-5 µm) and stained with H&E dye and examined under light microscopy.

### Statistical analysis

- Analysis done using the Graph pad prism 5.01 software.
- Between the groups, the data was compared using One way ANOVA test followed by post hoc Tukey's test.
- The level of significance for each comparison in the analysis was calculated at 0.05.

## Results

Pyloric ligation induced ulcer model

- **Ulcer index:** On comparison using one-way ANOVA, the mean ulcer index between the groups was significantly different ($P < 0.001$) (Table 2). On post hoc analysis, it was higher in all pyloric ligated groups than the normal control group. In all treatment groups, the mean ulcer index was significantly lower than the disease control group ($P < 0.001$). On comparison with ranitidine treated group, it was significantly higher in aqueous extract *Murraya koenigii* 200 mg/kg ($p = 0.002$) and 400 mg/kg ($p = 0.041$). The mean ulcer index in the groups treated with both doses of MKL were comparable ($p = 0.92$) as shown in table 3.
- **Percentage of ulcer protection:** Compared to the disease control group, the ulcer protection in ranitidine group was 64.12%; in groups treated MKL in doses of 200 and 400 mg/kg, it was 45.2% and 50.07%, respectively (Table 4)
- **Volume of gastric juice:** On comparison using one-way ANOVA, the mean volume of gastric juice between the groups was significantly different ($P < 0.001$) (Table 2). On post hoc analysis, volume was significantly higher ($P < 0.001$) in induced groups than the normal control group. It was significantly lower in the ranitidine ($P < 0.001$), *Murraya koenigii* 400 mg/kg ($p = 0.042$) treated groups than the disease control group. Compared to ranitidine treated group, the mean volume in MKL 200 mg/kg ($P < 0.001$) and 400 mg/kg ($p = 0.001$) was significantly higher (Table 3).
- **pH of gastric juice:** On comparison using one-way ANOVA, the mean pH of gastric juice between the groups was significantly different ($P < 0.001$) (Table 2). On post hoc analysis, the mean pH was significantly lower in the disease control ($P < 0.001$) and AEMK200 ($P < 0.001$) groups than normal control. The mean pH was significantly higher in the all treatment groups than the disease control group ($P < 0.001$). On comparison with ranitidine treated group, the mean pH of gastric juice in MKL 200 mg/kg ($P < 0.001$) treated groups was significantly lower. The mean pH in the group treated with MKL 400 mg/kg ($P < 0.001$) was significantly higher than the 200 mg/kg treated group (Table 3).
- **Free acidity of gastric contents:** On comparison using one-way ANOVA, it was observed that the mean free acidity of gastric juice between the groups was significantly different ($P < 0.001$)(Table 2). On post hoc analysis, it was observed that the mean free acidity of gastric juice was significantly higher ($P < 0.001$) in all induced groups than the normal control group. The mean free acidity of gastric juice was significantly lower in the all treatment groups than the disease control group ($P < 0.001$). On comparison with ranitidine treated group, the mean free acidity of gastric juice in aqueous extract MKL 200 mg/kg ($P < 0.001$) and 400 mg/kg ($P < 0.001$) treated groups was significantly higher. The mean free acidity of gastric juice in the group treated with aqueous extract MKL 400 mg/kg ($P < 0.001$) was significantly lower than the 200 mg/kg treated group (Table 3).
- **Total acidity of gastric contents:** On comparison using one-way ANOVA, it was observed that the mean total acidity of gastric juice between the groups was significantly different ($P < 0.001$) (Table 2). On post hoc analysis, it was observed that the mean total acidity of gastric juice was significantly higher ($P < 0.001$) in all induced groups than the normal control group. The mean total acidity of gastric juice was lower

| Groups | Ulcer index | Volume of gastric juice (ml) | Ph of gastric juice | Free acidity of gastric contents | Total acidity | Statistical | P value | Interpretation |
|---|---|---|---|---|---|---|---|---|
| NC | 0 | 1.14 ± 0.13 | 3.08 ± 0.11 | 10.02 ± 0.24 | 18.47 ± 0.38 | One way ANOVA | P < 0.001 | The mean ulcer index ,volume of gastric juice, ph of gastric juice, free acidity and total acidity of gastric juice between the groups was significantly different. |
| DC | 4.38 ± 0.43 | 3.31 ± 0.34 | 1.81 ± 0.09 | 30.94 ± 1.56 | 42.86 ± 0.58 | | | |
| R20 | 1.57 ± 0.32 | 2.45 ± 0.27 | 3.26 ± 0.11 | 11.48 ± 0.4 | 24.34 ± 1.09 | | | |
| AEMK200 | 2.4 ± 0.44 | 3.23 ± 0.14 | 2.54 ± 0.22 | 19.35 ± 0.64 | 32.6 ± 0.58 | | | |
| AEMK400 | 2.19 ± 0.37 | 2.96 ± 0.11 | 3.12 ± 0.09 | 16.28 ± 0.39 | 28.14 ± 0.88 | | | |

**Table 2:** Pyloric ligation model.

Effect on ulcer index, volume of gastric juice, Ph of gastric juice, free acidity of gastric juice and total acidity.

| Group 1 | Group 2 | Ulcer index | | Volume of gastric juice | | Ph of gastric juice | | Free acidity | | Total acidity | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P value | interpretation | P value | Interpretation | P value | interpretation | P value | interpretation | P value | interpretation |
| NC | DC | <0.001 | The mean ulcer index in the other groups was significantly higher than the NC group | <0.001 | The mean gastric juice volume in the other groups was significantly higher than the NC group. | <0.001 | The mean pH was significantly lower in the DC, AEMK200 group than NC, while it was comparable with NC group in R20, and AEMK400 groups. | <0.001 | The mean free acidity in DC, R20, AEMK200, AEMK400 groups was significantly higher than the NC group. | <0.001 | The mean total acidity in DC, R20, AEMK200, AEMK400 groups was significantly higher than the NC group. |
| | R20 | <0.001 | | <0.001 | | 0.309 | | 0.019 | | <0.001 | |
| | AEMK 200 | <0.001 | | <0.001 | | <0.001 | | <0.001 | | <0.001 | |
| | AEMK 400 | <0.001 | | <0.001 | | 0.998 | | <0.001 | | <0.001 | |
| DC | R20 | <0.001 | The mean ulcer index in R20, AEMK200, AEMK400 groups was significantly lower than the DC group | <0.001 | The mean gastric juice volume was significantly lower in the R20, AEMK400 group than DC group, while it was comparable with DC group in AEMK200 groups | <0.001 | The mean pH in R20, AEMK200, AEMK400 groups was significantly higher than the DC group. | <0.001 | The mean free acidity in R20, AEMK200, AEMK400 groups was significantly lower than the DC group. | <0.001 | The mean total acidity in R20, AEMK200, AEMK400 groups was significantly lower than the DC group |
| | AEMK200 | <0.001 | | 0.990 | | <0.001 | | <0.001 | | <0.001 | |
| | AEMK400 | <0.001 | | 0.042 | | <0.001 | | <0.001 | | <0.001 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| R20 | AEMK200 | 0.002 | The mean ulcer index in AEMK200 groups was significantly higher than the R20 group, while it was comparable with R20 group in AEMK-400groups. | <0.001 | The mean gastric juice volume was significantly higher in the AEMK200, AEMK400 than R20 group. | <0.001 | The mean pH in R20, AEMK200 groups was significantly lower than the R20 group, while it was comparable with R20 group in AEMK400 | <0.001 | The mean free acidity in AEMK200, AEMK400 groups was significantly higher than the R20 group. | <0.001 | The mean total acidity in AEMK200, AEMK400 groups was significantly higher than the R20 group. |
| | AEMK400 | 0.041 | | 0.001 | | 0.638 | | <0.001 | | <0.001 | |
| AEMK200 | AEMK400 | 0.920 | Mean ulcer index was comparable between AEMK200 and AEMK400 groups | 0.200 | Mean gastric juice volume was comparable between AEMK200 and AEMK400 groups. | <0.001 | Mean pH was significantly higher in AEMK400 group than AEMK200 group | <0.001 | Mean free acidity was significantly lower in AEMK400 group than AEMK200 group. | <0.001 | Mean total acidity was significantly lower in AEMK400 group than AEMK200 group. |

**Table 3:** Post hoc analysis using Tukey's test.

| Groups | Percentage of ulcer protection compared to disease control | |
|---|---|---|
| | Pyloric ligation method | Cold restraint method |
| NC | - | - |
| DC | - | - |
| R20 | 64.12% | 67.9% |
| AEMK200 | 45.2% | 47.49% |
| AEMK400 | 50.07% | 54.82% |

**Table 4:** Percentage of ulcer protection.

in the all treatment groups than the disease control group (P < 0.001). On comparison with ranitidine treated group, the mean total acidity of gastric juice in aqueous extract MKL 200 mg/kg(P < 0.001) and 400 mg/kg (P < 0.001) treated groups was significantly higher. The mean total acidity of gastric juice in the group treated with aqueous extract MKL 400 mg/kg (P < 0.001) was significantly lower than the 200 mg/kg treated group (Table 3).

### Cold Restraint stress induced ulcer model

- **Ulcer index:** On comparison using one-way ANOVA, it was observed that the mean ulcer index between the groups was significantly different (P < 0.001) (Table 5). On post hoc analysis, it was observed that in all induced groups, the mean ulcer index was significantly higher (P < 0.001) than the normal control group. The mean ulcer index in all treatment groups, the mean ulcer index was significantly lower than the disease control group (P < 0.001). On comparison with ranitidine treated group, the mean ulcer index in aqueous extract MKL 200 mg/kg (P < 0.001) and 400 mg/kg (p = 0.013) treated groups was significantly higher. The mean ulcer index in the groups treated with both doses of MKL were comparable (p = 0.4) (Table 6).
- **Percentage of ulcer protection:** Compared to the disease control group, the ulcer protection in ranitidine group was

67.9%; in groups treated with aqueous extract of MKL in doses of 200 and 400 mg/kg, it was 47.49% and 54.82%, respectively (Table 4).

| Groups | Ulcer index | Statistical test | F distribution and P value | Interpretation |
|---|---|---|---|---|
| NC | 0 | One way ANOVA | F (6,35)= 139.8 P < 0.001 | The mean ulcer index between the groups was significantly different. |
| DC | 3.76 ± 0.44 | | | |
| R20 | 1.21 ± 0.14 | | | |
| AEMK200 | 1.97 ± 0.26 | | | |
| AEMK400 | 1.7 ± 0.22 | | | |

**Table 5:** Cold restraint model.

| Group 1 | Group 2 | P value | Interpretation |
|---|---|---|---|
| NC | DC | <0.001 | The mean ulcer index in DC, R20, AEMK200, AEMK400 groups was significantly higher than the NC group. |
| | R20 | <0.001 | |
| | AEMK200 | <0.001 | |
| | AEMK400 | <0.001 | |
| DC | R20 | <0.001 | The mean ulcer index in R20, AEMK200, AEMK400 groups was significantly lower than the DC group. |
| | AEMK200 | <0.001 | |
| | AEMK400 | <0.001 | |
| R20 | AEMK200 | <0.001 | The mean ulcer index in AEMK200, AEMK400 groups was significantly higher than the R20 group |
| | AEMK400 | 0.013 | |
| AEMK200 | AEMK400 | 0.4 | Mean ulcer index was comparable between AEMK200 and AEMK400 groups. |

**Table 6:** Post hoc analysis using Tukey's test.

## Discussion

The present study demonstrated the antiulcer activity of aqueous extract of leaves of MKL in animal models of pyloric ligation and cold restraint induced gastric ulcers. MKL leaf extract has dem-

**Figure 1:** Histopathological images of gastric mucosa after Hematoxylin-eosin staining under light microscope (pyloric ligation ulcer model).

**Figure 2:** Histopathological images of gastric mucosa after Hematoxylin-eosin staining under light microscope (Cold restraint stress induced ulcer model).

onstrated antiulcer activity in some animal studies. These plants are easily available throughout the country in abundance, thus, giving us the opportunity to develop a cheap and easily available alternative for management of peptic ulcers. Thus, these plants were chosen and their antiulcer efficacy was evaluated in comparison to that with ranitidine.

In the present study, induction of ulcer by pyloric ligation or cold restraint stress was seen in the disease control groups, which was evident from the increase in ulcer index in comparison to the normal control group. Other variables also differed significantly.

### Pyloric ligation induced ulcer model findings

With treatment by aqueous extract of MKL, the ulcer index was lower compared to the disease control group, but higher compared to ranitidine. Dose dependent effect was not observed. The ulcer protection was 50% at 400 mg/kg dose and 45.2% at 200 mg/kg dose. The gastric juice volume was lower at 400mg/kg dose compared to disease control group. Compared to ranitidine, at both doses the gastric volume was higher. The gastric pH was increased compared to disease control group. In comparison to ranitidine, only 400 mg/kg dose gave comparable results. pH was higher compared to 200 mg/kg dose, representing a dose dependent effect. The free and total acidity of gastric contents was reduced than disease control group, however, the effect was not comparable to the ranitidine group, and also a dose dependent effect was observed with better response at higher dose. These results revalidate the findings of some other previous studies [13-16].

### Cold restraint stress induced ulcer model findings

The ulcer index in the aqueous extract of MKL at both doses was lower than the disease control group, but was higher than ranitidine group, and similar at both doses. The ulcer protection provided was lower than that by ranitidine treatment at both doses.

From the findings of the study, it was evident that the aqueous extracts of leaves of MKL have antiulcer activity. This activity measured in terms of ulcer index and ulcer protection was evident compared to the disease control group. Ulcer protection was found to be more at higher doses compared to the lower doses. The pH, volume, free and total acidity represent secretory activity in the stomach. The effect on these variables demonstrated antisecretory activity of the aqueous extract of MKL. Antiulcer activity of aqueous extract of *Murraya koenigii* leaves in the dose of 200 mg/kg and 400 mg/kg has been demonstrated in few studies. Patidar., *et al.* [17] and Sharma., *et al*. [18] reported antiulcer activity in terms of substantial ulcer percentage protection and changes in the volume, pH, free and total acidity of gastric juice as observed in number of ulcer models induced by various methods. Thus, the present study findings are in agreement with the previous studies evaluating the antiulcer activity of aqueous extract of leaves of MKL. A compound isolated from the leaves, 1,3,5-trihydroxy-7-methylanthracene-9,10-dione, has been found to be responsible for the antiulcer activity. However, the leaves contain numerous other phytoconstituents which have not yet been evaluated for their antiulcer potential.

## Conclusion

The present study adds to evidence of antiulcer efficacy of aqueous extracts of leaves of *Murraya koenigii*. Though not as good as ranitidine, it is a promising agent for antiulcer compounds. Research should be encouraged to identify and isolate the active phyto-constituents from the aqueous extracts of the leaves of these plants. The available evidence of antiulcer activity provides satisfactory evidence for further advanced studies with more pure and

refined products for testing its efficacy in human. Development of compounds as antiulcer agents may have an additional advantage of being cheap and easily available.

## Conflict of Interest

Nothing to declare.

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