



Received on 28 July, 2011; received in revised form 21 September, 2011; accepted 28 September, 2011

STUDIES ON ANTIULCER ACTIVITY OF ESSENTIAL OIL OF *CALAMINTHA OFFICINALIS* MOENCH.

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ABSTRACT

Keywords:

Calamintha officinalis Moench.,
Calamint,
Gastric ulcers,
Diclofenac sodium

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The present study was undertaken to determine the anti-ulcer potential of *Calamintha officinalis* Moench, a standardized oil of *Calamintha officinalis* Moench, commonly known as Calamint. Effect of various doses (0.4ml/kg and 0.8ml/kg,) of *Calamintha officinalis* Moench. oil was studied on gastric ulcers in pylorus ligation, Diclofenac sodium induced gastric mucosal injury in rats. Anti-ulcer activity was evaluated by measuring the ulcer index, gastric content, total acidity, and pH of gastric fluid. *Calamintha officinalis* Moench, dose dependently decreased gastric content, total acidity, ulcer index and increased pH of gastric fluid in pylorus ligation ulcer model. In Diclofenac sodium induced ulcer models, all the doses of *Calamintha officinalis* Moench. oil decreased the ulcer index and increased the pH gastric fluid. These results supported the ethnomedical uses of oil of *Calamintha officinalis* Moench. in the treatment of gastric ulcer.

INTRODUCTION: *Calamintha officinalis* (Moench) is a plant species belonging to the family Lamiaceae, similar to the common mints not only regarding its morphology but also in regard to its scent characteristics. This has led to it being used popularly as a substitute for the official mints in various beverages. Although similar pharmacological properties between these species could be speculated, in so far as we know the literature cites only chemical data regarding *C. officinalis* and other related species^{1, 2, 3}.

A peptic ulcer is a sore on the lining of the stomach or duodenum, the beginning of the small intestine. Less commonly, a peptic ulcer may develop just above the stomach in the esophagus, the tube that connects the mouth to the stomach.

A peptic ulcer in the stomach is called a gastric ulcer (fig. 1). One that occurs in the duodenum is called a duodenal ulcer. People can have both gastric and duodenal ulcers at the same time. They also can

develop peptic ulcers more than once in their lifetime. Peptic ulcers are common. Each year in the United States, about half a million people develop a peptic ulcer. A bacterium called *Helicobacter pylori* (*H. pylori*) is a major cause of peptic ulcers.

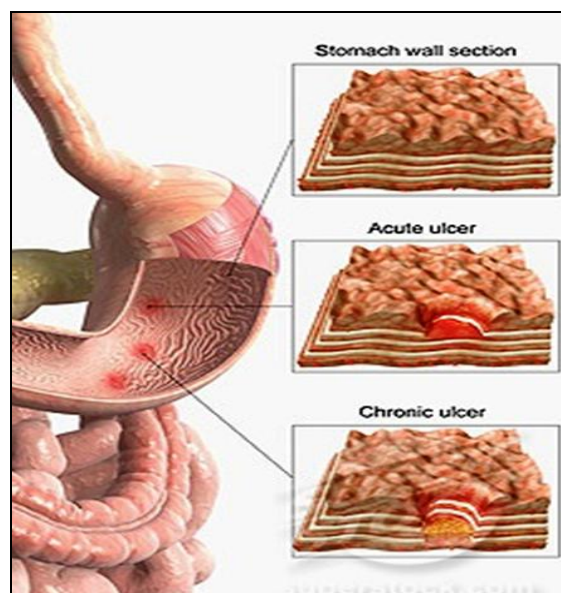


FIG. 1: TYPES OF GASTRIC ULCER

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen, are another common cause. Rarely, cancerous or noncancerous tumors in the stomach, duodenum, or pancreas cause ulcers. Peptic ulcers are not caused by stress or eating spicy food, but both can make ulcer symptoms worse. Smoking and drinking alcohol also can worsen ulcers and prevent healing^{4,5,6}.

MATERIALS AND METHODS:

Plant material: The plant material was collected from Bareilly, Uttar Pradesh, India, during the month of Sept. 2010 and air-dried. The plant for the proposed study was identified and authenticated by taxonomist Dr. Gaurav Nigam, Associate Prof., Department of Botany, Bundelkhand University, Jhansi (U.P.), India, Voucher specimen (No. 922) has been deposited in the herbarium of the same department.

Animals: Healthy Wistar Albino rats, weighing about (180-250gm) of either sex were obtained from animal house, Institute of Pharmacy, Bundelkhand University, Jhansi. The animals were housed under the uniform laboratory condition & fed with commercial diet & provide with water ad libitum, during the experiment. The animal were procured from Gwalior & permitted for study under the Institutional Animal Ethical Committee. The reference number of Institutional animal ethical committee is BU/PHARM/IAEC/04.

Essential Oil: Fresh samples of the plant leaves of *Calamintha officinalis* Moench. (Lamiaceae) were distilled in Clavenger apparatus over 6 hrs. The essential oil obtain was dried with anhydrous sodium sulphate and stored in vacuum tight container at 4°C. The essential oil collected was light yellow in color, having a strong pleasant odor. Its extractive value was found to be 9.44%v/w⁷.

Acute Oral Toxicity Studies: Acute oral toxicity was performed by using OECD guidelines-423 (Organization of Economic Co-Operation Development) - Fixed Dose Procedure. The purpose of this study is to allow selection of the appropriate starting dose for the main study. Acute oral toxicity studies of *Calamintha officinalis* Moench. was performed in Swiss Albino mice. The mice were kept for 4 hr of fasting prior to the experiment and body weight of the mice should be

noted. Usually mice weighting 25-35 gm were used for acute toxicity studies. The dose was given to every mice orally according to body weight. The test for acute toxicity was performed at 0.25, 0.50, 0.75, and 1.0 ml/kg oral dose of Ethanolic extract of aerial part of *Calamintha officinalis* Moench.

Food was given for a 1-2 hours after the administration of drug. During the first 4 hr. after the drug administration, animals were continuously observed for gross behavioral changes & then observation is continued for 24 hr & 72 hr in regular intervals for 14 days. The parameter such as hyperactivity, grooming, convulsions, sedation, hypothermia, change in fur colour, mortality or moribund stage or death was observed (**table 1**).

TABLE 1: ASSESSMENT OF ACUTE TOXICITY STUDIES

Group	Dose	No. of mice	Mortality	
			24 hrs	72 hrs
Extracted dose of oil	0.25 ml	6	0	0
	0.50 ml	6	0	0
	0.75ml	6	0	0
	1.0ml	6	0	0

RESULT: LD₅₀ of extracted oil of aerial part of *Calamintha officinalis* Moench. was done as per OECD guidelines (Revised draft 423). The animal did not show any signs of toxicity and behavioral changes upto 1.0 ml/kg dose after 24 hrs and 72 hrs.

Evaluation of Antiulcer Activity: Two models (Diclofenac sodium and Pylorus ligation induced ulcer) were employed experimentally in rats to evaluate the antiulcer activity of the extracted oil of *Calamintha officinalis* Moench.

Diclofenac Sodium Induced Ulcer Model : All the animals were grouped into five groups. Each group had 3 animals.

- Group - 1 was negative control (without any treatment fed with normal water).
- Group - 2 was positive control treated with NSAID.
- Group - 3 had pretreated animals with 0.4 ml/kg dose of extracted oil and then treated with NSAID.

- Group - 4 was with pretreated animals with 0.8 ml/kg dose of extracted oil and then treated with NSAID.
- Group - 5 was standard control pretreated with Ranitidine and then treated with NSAID following the dose and mode of administration.

All the animals were by oral gavages with the help of feeding tube. The doses determined as low dose at the rate of 0.4 ml/ kg of the body weight and high dose at the rate of 0.8 ml/kg of the body weight for both the sample. Then non steroidal anti- inflammatory drug, diclofenac sodium was used as the ulcerogenic agent at the dose of 100 mg/kg of body weight. Standard anti ulcer drug ranitidine used at the rate of 20 mg/kg of body weight. *In vivo* Protocol all the groups of animals were kept overnight fasting, fed only with the tap water.

The animals of group III, IV were treated with the sample extracted oil at different doses. This treatment was given thrice at the 12 hours interval. Animals of the group V were treated with ranitidine simultaneously. After one hour of last administration of sample extracted oil the NSAID was given by oral gavages to the group II to group V animals.

TABLE 2: ULCER SCORE AT DIFFERENT CONCENTRATION OF CALAMINTHA OFFICINALIS OILS

Group	Group name	Concentration	Ulcer Score (Mean ± SD)	Volume of gastric juice	pH of gastric juice
1	Negative control	-----	-----	-----	-----
2	Positive control	100 mg/kg	3.23±0.03	1.52	2.7
3	Dose of oil	0.4 ml/kg	2.48±0.01	0.88	3.0
4	Dose of oil	0.8 ml/kg	3.36±0.01	1.28	4.9
5	Standard control	20.0 mg/kg	1.50±0.01	1.19	5.9

RESULT: Diclofenac sodium caused damage on the glandular mucosa (3.23±0.03). In contrast to it, the pretreatment with extracted oil of *Calamintha officinalis* Moench. at doses at 0.4 ml/kg and 0.8 ml/kg of the body weight decreased the ulcerated area to 2.48±0.02 and 3.36±0.01, respectively, which was comparable to the effect exerted by Ranitidine 1.50±0.01.

Administration of oil of *Calamintha officinalis* Moench., decreased the gastric volume in comparison with rats treated with ranitidine. The gastric volume gets decreased, simultaneously the gastric acidity also decreased significantly.

After 6 hours of NSAID administration, the animals were sacrificed by cervical dislocation. The animals were dissected and the stomach was taken out. Finally the ulcers were observed macroscopically. The observation was made for any bulging or inflammation in the stomach. The stomachs were opened along the greater curvature and the mucosa was exposing for evaluation. The ulcer scores (US) were calculated as the arithmetic mean for each treatment given in **table 2**. Sacrifying the rat, stomach was removed and opened along the greater curvature and washed it slowly under top water, but it on the glass slide and observed naked eye⁸.

$$\text{Ulcer index} = \frac{\text{Area of ulcer}}{\text{Total stomach area}} \times 100$$

Collection of Gastric Juice: The stomach was carefully keeping the esophagus closed opened along the greater curvature and the gastric contents were removed the gastric contents were collected in plain tubes and centrifuged at 3000 rpm for 5 min the volume of the supernatant was expressed as ml/100 gm body weight the mucosa was flushed with saline and observed for gastric lesions using the macroscopic structure.

Pylorus ligated Ulcer Technique: Pylorus ligation was done by ligating the pyloric end of the stomach of rats 1 h after test substance administration. Animals were allowed to recover and stabilized in individual cage and were deprived of water during postoperative period.

After 4 h of surgery, rats were sacrificed and gastric juice was collected, centrifuged and the supernatant was measured. Gastric contents were analysed for total acidity by titrating against 0.01 N NaOH using phenolphthalein as indicator. The pH of gastric juice was measured using pH meter. The ulcers were examined on the dissected stomachs as described below in **table 3** and ulcer index was measured⁹.

TABLE 3: ULCER INDEX AT DIFFERENT CONCENTRATION OF *CALAMINTHA OFFICINALIS* OIL

Group	Treatment	Dose	Gastric content pH (mg/kg)	pH	Total acidity (mEq/L/100 g)	Ulcer index
1	Control	-----	-----	-----	-----	-----
2	Pylorus control	100mg/kg	6.39±0.01	2.13±0.01	124.73±0.03	3.62±0.05
3	Standard control	20mg/kg	2.51±0.01	6.79±0.01	16.72±0.01	30.34±0.03
4	CO 1	0.4ml/kg	3.76±0.01	2.49±0.01	93.17±0.02	3.44±0.03
5	CO 2	0.8ml/kg	3.53±0.01	2.58±0.02	82.77±0.01	6.97±0.01

RESULTS: The results obtained when ulceration of the gastric mucosa was induced by pylorus ligation were shown in above table. In gastric secretion studies, it was found that pylorus ligation control rats having the gastric pH of 2.13±0.01 was increased when treated with *Calamintha officinalis* Moench. in a dose-dependent manner. Ranitidine, the standard control showed highest gastric pH (6.79±0.01). The pylorus ligation control animals showed total acidity of 124.73±0.03 mEq/L/100g, whereas *Calamintha officinalis* Moench. decreased the total acidity in a dose dependent manner.

Total acidity was significantly decreased at 0.8 ml/kg dose of *Calamintha officinalis* Moench. Oil. The gastric content of pylorus ligation control animals were 6.39±0.01 ml, whereas *Calamintha officinalis* Moench. significantly decreased the gastric content in a dose dependent manner. In measurement of ulcer index, the pylorus ligation control animals showed ulcer index of 3.62±0.05 whereas *Calamintha officinalis* Moench. decreased the ulcer index significantly in a dose-dependent manner.

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