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A. R. Mullaicharam
 Department of Pharmacy
 Oman Medical College
 Sultanate of Oman.

M. Surendra kumar
 Sastra university, Thanjavur, Tamil
 nadu, South India

Effect of *Michelia champaca* Linn on pylorous ligated rats

A. R. Mullaicharam, M. Surendra kumar

ABSTRACT

Michelia champaca Linn (Magnoliaceae) is used in folk medicine for the treatment of ulcers and it is main species of *Michelia* used for the treatment of gastric ulcers. However, there have been no reports regarding the anti-ulcer activity of *Michelia champaca* Linn extracts. Therefore, this study was carried out to evaluate the anti-ulcerogenic property of the alcoholic and aqueous extracts of leaves and flowers of *Michelia champaca* Linn. The anti-ulcer assays were performed using nonsteroidal anti-inflammatory drug (NSAID)- aspirin induced ulcer. The effects of the extracts on gastric content volume, pH, total acidity, ulcer index and stomach using the pylorus ligated model were evaluated at a dose of 300mg/kg. In the aspirin induced ulcer model, it was observed that the treatment with *Michelia champaca* Linn extracts significantly reduced the gastric juice, total acidity, ulcer index and elevation in gastric pH. The results were comparable with the positive control cimetidine 50mg/kg. Further the results were confirmed using histopathological studies of the stomach. These results showed the anti-ulcerogenic property of *Michelia champaca* Linn extracts. The maximum efficacy was shown by the flower aqueous extract followed by leaf alcoholic, flower alcoholic and leaf aqueous extracts.

Key words: *Michelia Champaca* Linn , Pylorous Ligation, Antiulcer.

INTRODUCTION

Michelia champaca Linn (Panda et al, 2005; Nadkarni et al, 1996; Warrie et al ,1995) (Magnoliaceae) is a taxon of dicotyledonous flowering trees. The tree bears large cup shaped orange flowers with fragrant aroma. The plant is a very good source of esters of benzoic acid, benzaldehyde, benzyl alcohol, isoeugenol and sesquiterpene lactones. A rapid progress in the field of gastroenterology had led to the identification of several potential supportive drugs from phytomedicine that are becoming part of integrative health care system of industrialized nations. In our current search for phytomedicine, we found that various parts of *Michelia champaca* Linn have been widely used for anti-inflammatory, anti-pyretic, anti-microbial, cardiotoxic, purgative, diaphoretic, stimulant, diuretic and anti-leprotic properties. Literature survey revealed that *Michelia champaca* Linn has been reported to contain michelia - A, lirioidenine, parthenolide and guaianolides (Kazuoto et al ,1963 ;Majumder et al, 1963; Benerjee et al, 1964; Banerjee et al, 1964 ; Hoffmann et al, 1977; Toshiyuki et al, 1982). The results of preliminary phytochemical studies revealed the presence of flavonoids suggesting its anti-oxidant property. This property could contribute to the observed anti-ulcer effect by *Michelia champaca* Linn (Mallika et al, 2006).

MATERIALS AND METHODS

Plant Material and Extraction

The leaves and flowers used in this study were procured from the local areas of Coimbatore, Tamil Nadu, India and authenticated with the standard sample in the Botanical Survey of India, Coimbatore, Tamil Nadu by Dr. G.V.S. Murthy. The dried leaves and flowers were coarsely powdered and weighed quantity of this material were taken and divided into two

***For Correspondence:**
Dr. A. R. Mullaicharam
 Department of Pharmacy
 Oman Medical College
 Sultanate of Oman.
 Email: mullaicharam@yahoo.com.

portions. First portion was extracted with water by means of maceration technique using chloroform as preservative (0.3%). The second portion was extracted using 95% ethanol in a soxhlet extractor. The last traces of solvents were removed under vacuum drier and the solid residue was stored in a refrigerator until further use.

The percentage yield of leaf ethanolic and aqueous extracts were 22.80 % w/w and 24.54 % w/w respectively and the percentage yield of flower ethanolic and aqueous extracts were 23.50 % w/w and 28.99 % w/w respectively.

Phytochemical Analysis

The chemical constituents of the extracts were identified by means of qualitative chemical tests (Mukherjee et al, 2002; Khandelwal et al ,2004; Khandelwal et al, 1996; Finar et al, 1975; Kokate et al, 1994; Evans et al, 1994; Jackson et al, 1986) and further confirmed by Thin Layer Chromatography according to standard procedures (Wagner et al, 1984).

Animals

Adult male albino rats of Wistar strain weighing 200-250gms were used and divided into 6 groups with 6 animals each. The animals were housed at standard laboratory conditions and fed with standard laboratory food and provided with water ad libitum.

Experimental method

All the animals were given 200mg/kg aspirin in 1% CMC solution along with their respective treatments using oral route for 5 days. Group I animals received 1 ml of 0.9% saline. Group II animals received cimetidine 50mg/kg, Group III received flower aqueous extract 300mg/kg, Group IV received flower alcoholic extract 300mg/kg. Group V received leaves aqueous extract 300mg/kg where as Group VI received leaves alcoholic extract 300mg/kg.

On the 6th day pylorus ligation was made as per the procedure using ether anesthesia. The animals were sacrificed after 4h, abdomen was opened and the stomach was isolated. The gastric juice was collected in a measuring cylinder and the stomach was opened along the curvature. The mucosa was washed with 1ml distilled water and the washings were added to the gastric juice, the gastric contents were centrifuged at 1000 rpm for 10minutes. 1 ml of the supernatant was diluted with 9ml of distilled water. The solution was titrated against 0.01N sodium hydroxide using phenolphthalein as indicator (Kulkarni et al, 1993; Tandon et al, 2004; Goel et al, 2002; Anagha et al, 1998; Maity et al, 2003; Pandit et al, 2000; Mandal et al, 1998; Goswami et al, 1998; Suleyman et al, 2001; Mohan et al, 1988; Hitner et al, 2001; Chanda et al, 2002; Gerhard et al, 2000).

Calculations

$$\text{Total Acidity} = \frac{\text{Titrate value} \times \text{Normality of Sodium Hydroxide} \times 100}{0.1}$$

The Ulcer scoring was done as follows:

- 0.0= Normal Mucosa
- 0.5= Red Coloration
- 1.0= Spot ulcers
- 1.5= Hemorrhagic streaks
- 2.0= Ulcers >3 but <5
- 3.0= Ulcers > 5

The mean of the ulcer scores in each group was taken as ulcer Index.

Histological Studies

The stomach from each groups were fixed in 10% formalin for 24h. The fixative was removed by washing through running tap water over night. After dehydration through graded series of alcohols, the tissues were cut into 3-5 μ m thickness and stained with haemotoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under light microscope for details.

Statistical Analysis

Data are reported as mean \pm standard error of the mean (S.E.M.) and were compared using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison test and p values <0.05 were considered significant.

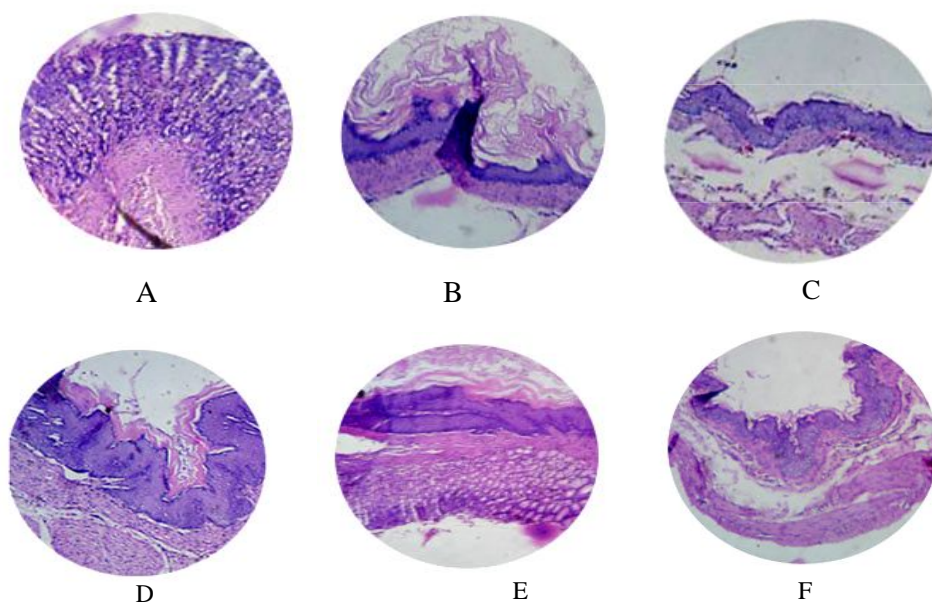
RESULTS

Phytochemical screening of the extracts

The leaf alcoholic extract showed the presence of alkaloids, flavonoids, glycosides and essential compounds such as carbohydrates and proteins along with tannins and sterols, Whereas the leaf aqueous extract was devoid of alkaloids, carbohydrates, glycosides, vitamin C and sterols. Both leaf alcoholic and aqueous extract does not have triterpenoids and Saponins. Similarly the phytochemical screening of flower alcoholic extract showed the presence of alkaloids, flavonoids and glycosides. The essential components such as carbohydrates, proteins and tannins were also present. The flower aqueous extract showed the presence of flavonoids, carbohydrates, proteins and tannins. The flower and leaf alcoholic extract were identical in bearing the same phytoconstituents.

Effect of leaf extracts (Alcoholic and Aqueous) of *Michelia champaca* Linn on ulcer index in pylorus ligation of rats

Administration of leaf alcoholic and aqueous extracts at the dose of 300mg/kg for consecutive 5 days was done by oral route. On the 6th days 4h after pylorus ligation the amount of gastric juice was 2.77ml and 3.2ml of the aqueous and alcoholic extract respectively, whereas the amount of gastric juice collected after the administration of aspirin and cimetidine was found to be 4.5ml and 1.6ml respectively. The leaf alcoholic extract showed significant (P<0.001) decrease in the volume of gastric juice when compared to only aspirin treated groups. Significant decrease was observed when leaf aqueous extract (P<0.001) and cimetidine (P<0.001) to the only aspirin treated group. The leaf



A. Histopathology of Stomach for negative control group (treated only with Aspirin) showing the occurrence of Ulcer with destruction over the Columnar epithelial layer and Lamina propria region. **B.** Histopathology of Stomach for Positive control group (treated with Aspirin and Cimetidine) showing the Prevention of occurrence of Ulcer with more or less normal atrophy of the tissue. **C.** Histopathology of Stomach for flower aqueous extract (treated with Aspirin and Flower aqueous extract) showing the prevention of occurrence of Ulcer with normal make up of Columnar epithelial cell with slight destruction over the lamina propria region. **D.** Histopathology of Stomach for flower alcoholic extract (treated with Aspirin and Flower alcoholic extract) showing the prevention of occurrence of Ulcer with normal make up of Columnar epithelial cell with slight destruction over the lamina propria region. **E.** Histopathology of Stomach for Leaf aqueous extract (treated with Aspirin and Leaf aqueous extract) showing the very poor prevention of occurrence of Ulcer with damaged Columnar epithelial cells and with slight destruction over the lamina propria region. **F.** Histopathology of Stomach for Leaf alcoholic extract (treated with Aspirin and Leaf alcoholic extract) showing the prevention of occurrence of Ulcer with slight alteration over the make up of Columnar epithelial cell with slight destruction over the lamina propria region.

alcoholic extract (28 mEq/l), ($P < 0.01$) and cimetidine (14 mEq/l), ($P < 0.001$) treated groups showed significant decrease in the total acidity when compared to only aspirin (34 mEq/l) treated group.

The leaf aqueous ($P < 0.001$) and alcoholic extract ($P < 0.001$) showed significant decrease in ulcer index (1.5 and 1.0,) respectively as compared to only aspirin (3.0) treated group. The ulcer index of the cimetidine treated group was found to be 0.1 ($P < 0.001$).

Effect of flower extracts (Alcoholic and Aqueous) of *Michelia champaca* Linn on ulcer index in pylorus ligation of rats:

Administration of flower alcoholic and aqueous extracts at the dose of 300mg/kg for consecutive 5 days was done by oral route. On the 6th day 4h after pylorus ligation the amount of gastric juice was 2.96ml and 2.5ml of the aqueous and alcoholic extract respectively. Where as the amount of gastric juice collected after the administration of aspirin and cimetidine was found to be 4.5ml and 1.6ml respectively. The flower alcoholic extract showed significant ($P < 0.001$) decrease in the volume of gastric juice when compared to only aspirin treated groups. Significant decrease was observed when flower aqueous extract ($P < 0.001$) and cimetidine ($P < 0.001$) to the only aspirin treated group. The flower aqueous extract (23 mEq/l), ($P < 0.001$) and cimetidine (14 mEq/l), ($P < 0.001$) treated groups showed significant decrease in the total acidity when compared to only aspirin (34 mEq/l) treated group. The flower aqueous ($P < 0.001$) and alcoholic extract ($P < 0.001$) showed significant decrease in ulcer index (0.6 and 1.2)

respectively as compared to only aspirin (3.0) treated group. Where as the ulcer index of the cimetidine treated group was found to be 0.1 ($P < 0.001$).

DISCUSSIONS

There are several factors that may induce ulcer in human beings, such as stress, chronic use of anti-inflammatory drugs and continuous alcohol ingestion, among others. Although in most cases the etiology of ulcer is unknown, it is generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism. The candidate for an effective drug against peptic ulcer should basically act either by reducing the aggressive factors on gastroduodenal mucosa or by increasing mucosal resistance against them.

A thorough literature review on *Michelia champaca* Linn showed that it was not tried out for any anti-ulcer effect. The phytochemical screening of *Michelia champaca* Linn showed the presence of flavonoids, tannins and proteins in the leaves (alcoholic and aqueous) and flower (alcoholic and aqueous) extracts. Leaf alcoholic extract showed the presence of alkaloids, glycosides, and sterols. The presence of carbohydrate was observed in the leaf alcoholic extract as well as flower aqueous and alcoholic extracts. The presence of flavonoids was ascribed to the *Michelia* species and it is well known that many flavonoids display anti-secretory and cytoprotective properties in different experimental models of gastric ulcer. In addition, oxidative damage is considered to be a common factor in the pathogenesis of ulcers by

different experimental and clinical models. This antioxidant activity has been described for extracts containing flavonoids. Therefore, the anti-ulcer activity of *Michelia champaca* Linn extracts may be partially due to its relative antioxidant activity. Nonsteroidal anti-inflammatory drugs (NSAIDS), like aspirin and indomethacin, are known to induce ulcers during the course of

anti-inflammatory therapy, by inhibiting prostaglandin synthetase through the cyclooxygenase pathway. In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair. Thus, the suppression of prostaglandin synthesis by NSAIDS results in increased susceptibility to mucosal injury and gastroduodenal ulceration.

Preliminary studies were carried out to fix the dosage of extracts. 300mg/kg was observed to be the most effective dose which was then used for the study. In this work, we evaluated the activity of *Michelia champaca* Linn leaf (alcoholic & aqueous) and flower (alcoholic & aqueous) extracts on gastric secretion in the ligature pylorus model. A significant decrease in gastric fluid volume, and a decrease in acid output with an elevation in gastric pH, were observed after oral administration of *Michelia champaca* Linn extracts, when compared with the respective control, indicating that it possess anti-secretory potency. It was observed that *Michelia champaca* Linn flower aqueous extract displayed significant reduction of the mucosal damage in the aspirin-induced ulcer model followed by leaf alcoholic, flower alcoholic and leaf aqueous extracts. These results suggest the possible involvement of prostaglandins and/or mucus in the anti-ulcer effect of the extract.

In conclusion, the results of the present study show that *Michelia champaca* Linn flower and leaf(alcoholic & aqueous) extracts displays gastroprotective activity, as demonstrated by its significant inhibition of the formation of ulcers induced using different models, as well as its ability to decrease gastric secretions.

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Table: 1 Phytochemical screening of *Michelia champaca* Linn extracts

S.N	Phytoconstituents	Alcoholic Extract	Aqueous Extract
LEAF			
01	<i>Alkaloids</i>	+	-
02	Flavonoids	+	+
03	Glycosides	+	-
04	<i>Carbohydrates</i>	+	-
05	Proteins & Amino Acids	+	+
06	Vit.C	-	-
07	Tannins	+	+
08	Sterols	+	-
09	Triterpenoids	-	-
10	Saponins	-	-
FLOWER			
01	<i>Alkaloids</i>	+	-
02	Flavonoids	+	+
03	Glycosides	+	-
04	<i>Carbohydrates</i>	+	+
05	Proteins & Amino Acids	+	+
06	Vit.C	-	-
07	Tannins	+	+
08	Sterols	+	-
09	Triterpenoids	-	-
10	Saponins	-	-

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