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Anti-ulcer activity of *Cestrum nocturnum* leaf in ethanol- and indomethacin-induced ulcer models

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Abstract

This study was planned to explore the antiulcer activity of the methanolic and *n*-hexane extracts of *Cestrum nocturnum* leaf against ethanol- and indomethacin-induced ulcer models in rats. The rats were administered orally vehicle in normal control group, methanolic and *n*-hexane extracts at doses 300, 500, 700 mg/kg, in treated groups and omeprazole 20 mg/kg, as standard drug. The gastric tissues/contents were examined to determine the ulcer index, antiulcer activity, gastric pH, gastric juice volume and acidity. Both extracts showed dose-dependent increase in antiulcer activity (%) in both ulcer models. Histopathology also supported these results. Gastric pH significantly increased while the gastric juice and acidity significantly decreased in the treatment groups of both ulcer models indicating the anti-secretory effect of extracts. It may be concluded that *C. nocturnum* protect gastric mucosa by decreasing gastric juice, acidity and increasing the gastric pH.

Introduction

In clinical practice peptic ulcer is the most common gastrointestinal disorder (Bafna and Balaraman, 2004). In such cases both synthetic and herbal medication are better and newer option for management and treatment of peptic ulcer disease (Dharmani et al., 2005). The patho-physiological causes of gastric ulcer were not completely defined but reasons behind ulcer are imbalance between the physiological and aggressive factors (pepsin and acid secretion) and gastric mucus membrane cytoprotective factors (bicarbonates and mucus secretion). Several endogenous factors of ulcer protection are also involved, which includes somatostatin, prostaglandins E₂ sulfhydryl compounds and nitric oxide (de Sousa Araújo et al., 2008). Commercially available, antacids, H₂ blockers, proton pump inhibitors are standard therapies for ulceration. But the use of these drugs is associated with number of side effects. WHO (1980) has suggested the research on natural products in those conditions where the use of synthetic

drugs are not free from adverse effects. This recommendation creates a chance of discovering new therapeutic agents that are safe, less toxic and more efficacious in the treatment or prevention of ulcer from the community of medicinal plants.

Phyto-therapeutic agents are under limelight for the management and treatment of diseases globally (Cipriani et al., 2008; Gul et al., 2015). Number of plants have been investigated for anti-ulcer activity by number of researchers such as *Mentha longifolia*, *Dalbergia sissoo*, *Morus nigra*, *Convolvulus arvensis* and *Chenopodium murale* (Saleem et al., 2014; Mallhi et al., 2014; Ali et al., 2013; Gul et al., 2015; Gul et al., 2016).

Cestrum nocturnum belongs to solanaceae family with common name of "night blooming jasmine" (Patil et al., 2011). It is perennial shrub and pollinated with the help of night flying insects and attracted by its fragrance (Sykorova et al., 2003).

Cestrum nocturnum is being used as folklore remedy for



gastric ulcer. The present investigation was undertaken to provide scientific-based evidence to justify the folklore use as rational or irrational. Additionally the aim was to explore the cheap, easily available and having fewer side effects remedy for gastric ulcer.

Materials and Methods

Laboratory animals

Wistar albino rats between weights 150-200 g were purchased from University of Health Sciences-Lahore and accommodated at animal house of Faculty of Pharmaceutical Sciences Government College University Faisalabad, Pakistan. Rats were placed at the room temperature $22 \pm 2^\circ\text{C}$ and 12/12 period of light and dark with proper ventilation facility. The relative humidity 44 to 56% was maintained. Rats of different group were placed in different cages having wire bottom to avoid the coprophagy and to facilitate the accurate evaluation. Proceeding to the experimentation, for one week rats were acclimatized and given the standard diet and water *ad libitum*. The experiments were performed by the approval of Animal Ethics Committee of Government College University of Faisalabad, Pakistan.

Collection and identification of plant

Fresh leaves of *C. nocturnum* was collected from the botanical garden of University of Agriculture, Pakistan. The plant leaves were identified and authenticated by the taxonomist at the botany department of University of Agriculture, Pakistan. Leaves were separated, washed with water and dried under shade. Dried leaves were ground to fine powder.

Preparation of extract

C. nocturnum leaf extract was prepared by two methods: a) Maceration; b) Decoction.

Maceration

Leaves powder was soaked in methanol (1:4) for five days with vigorous shaking at regular time intervals. *n*-hexane extract of leaves was prepared similarly. Extract was filtered with Whatman filter paper No. 1 and excess of solvent was removed with rotary evaporator.

Decoction

Freshly taken leaves of *C. nocturnum* (150 g) were boiled in one liter of water for the five minutes. Then decoction was allowed to cool for the 30 min and filtered through the Whatman filter paper No. 1. A fresh decoction was prepared after every three days.

Study design

Two ulcer models were used in this study which is as follows:

Ethanol-induced gastric ulcer model

The rats were separated into four groups, then group IV sub-divided into seven sub-groups, each of contain five animals and kept on fasting for the 24 hours with water *ad libitum*. Group I normal control received normal saline according to weight, Group II disease control, Group III standard control treated with omeprazole (20 mg/kg, orally), Group IV (A, B, C, D, E, F and G) treated control administered methanolic and *n*-hexane extracts at doses 300, 500, 700 mg/kg, orally, decoction at dose (10 mL/kg, orally). After one hour dosing, ulcer was induced by administering ethanol (1 mL/kg, orally) to all groups except normal control. Animals were sacrificed by cervical dislocation, one hour after the administration of ethanol. Rats stomachs were removed, opened along the greater curvature, pinned on the soft board and stomach lesions were evaluated to determine the ulcer score.

Indomethacin-induce gastric ulcer model

The rats were separated into four group, then group IV sub-divided in seven sub-groups, each group contain five animals (n=5) and kept on fasting for 24 hours with water *ad libitum* before study. Group I normal control, Group II disease control, Group III standard control received omeprazole (20 mg/kg, orally), Group IV (A, B, C, D, E, F and G) treated with methanolic and *n*-hexane extracts at doses 300, 500 and 700 mg/kg, orally respectively and decoction at dose (10 mL/kg, orally). After one hour dosing, ulcer was induced by administration of indomethacin (30 mg/kg, orally) to all animals except normal control animal. Animals were sacrificed by cervical dislocation, one hour after administration of indomethacin. Rat stomachs were removed, opened along the greater curvature, pinned on the soft board and stomach lesions were evaluated to determine the ulcer score.

Ulcer index calculation

The number of ulcers was counted by using the magnifying glass. Severity scores (Nwidu and Nwafor, 2009; Patidar, 2011) are as follows:

Condition of stomach	Ulcer score
Normal coloration	0
Spot hemorrhage	0.5
Ulcer spots more than two	1.0
Ulcer lesions <3 mm	2.0
Ulcer covers more than 50% of stomach area	3.0
Lesions on 80% area	4.0
Perforations	5.0

$$\text{Ulcer index (UI)} = \frac{\text{Ulcer score}}{10}$$

Anti-ulcer activity (%) calculation

Anti-ulcer activity(%) /percentage protection is calculated by using following formula (Bhardwaj et al., 2012):

$$\text{Anti - ulcer activity} = \frac{\text{UI (disease control)} - \text{UI (Treated)}}{\text{UI (disease control)}} * 100$$

Gastric volume, pH and acidity were determined by adopting Reignier et al., 2013, Delgado-Aros et al., 2002 and Segal et al., 1950 methods respectively.

Histopathological evaluation of gastric tissues

The wall of sacrificed rat stomach was fixed in 10% formalin and then paraffin blocks were made. Five micrometer thick section was cut from the paraffin block and stained with hematoxylin and eosin. Histopathological evaluations were carried out on the collected gastric tissue samples according to the method as reported elsewhere (Yu et al., 2015).

Statistical analysis

One-way ANOVA followed by Dunnett's posthoc by using GraphPad Prism version 5 was used for statistical analysis. Data were presented as mean \pm SEM. $p \leq 0.05$ was taken as statistically significant value.

Results

Ulcer score, ulcer index and anti-ulcer activity (%) for ethanol- and indomethacin-induced ulcer models are given in Table I. In ethanol-induced ulcer models, there was dose-dependent increase in anti-ulcer activity in all treated groups. Methanol and *n*-hexane extract (700 mg/kg) showed 81% anti-ulcer activity which was higher than the standard value i.e. 75%. Decoction showed only 56% anti-ulcer activity. Indomethacin-induced ulcer model also showed identical results.

Table II shows the effect of extracts on the gastric pH, acidity and juice. In both ethanol- and indomethacin-induced ulcer models, both extracts at doses 300, 500, 700 mg/kg, orally, leaves decoction 10 mL/kg, orally and standard drug 20 mg/kg, orally significantly ($p \leq 0.05$) reduced the free acidity, total acidity and volume of gastric juice but significantly increased the pH of gastric volume in a dose-dependent manner when compared with the disease control group.

Histopathological evaluation

Histopathological assessment of the tissues of gastric mucosa of rats had also established the fact that

Table I

Ulcer score, ulcer index and anti-ulcer activity of *Cestrum nocturnum* extracts

Groups	Treatment	Dose	Ulcer score	Ulcer index	Anti-ulcer activity (%)
Group I (Normal control)	Normal saline	2 mL/kg			
Group II (Disease control)	Ethanol	1 mL/kg	15.5 \pm 0.0	1.6 \pm 0.0	0.0 \pm 0.0
	Indomethacin	30 mg/kg	14.5 \pm 0.0	1.5 \pm 0.0	0.0 \pm 0.0
Group III (Standard)	Omeprazole	20 mg/kg	3.5 \pm 0.2 ^a	0.4 \pm 0.0 ^a	75 \pm 4.1 ^a
Group IV (Ethanol-induced ulcer model)	Methanolic extract	300 mg/kg	5.6 \pm 0.5 ^a	0.6 \pm 0.1 ^a	69 \pm 3.3 ^a
	Methanolic extract	500 mg/kg	4.9 \pm 0.4 ^a	0.5 \pm 0.0 ^a	71 \pm 1.6 ^a
	Methanolic extract	700 mg/kg	2.9 \pm 0.5 ^a	0.3 \pm 0.1 ^a	81 \pm 3.4 ^a
	<i>n</i> -Hexane extract	300 mg/kg	4.9 \pm 0.4 ^a	0.5 \pm 0.0 ^a	69 \pm 2.4 ^a
	<i>n</i> -Hexane extract	500 mg/kg	4.1 \pm 0.5 ^a	0.4 \pm 0.1 ^a	75 \pm 3.2 ^a
	<i>n</i> -Hexane extract	700 mg/kg	2.6 \pm 0.4 ^a	0.3 \pm 0.0 ^a	81 \pm 2.4 ^a
	Decoction	10 mL/kg	7.0 \pm 1.2 ^a	0.7 \pm 0.1 ^a	56 \pm 2.6 ^a
Group V (Indomethacin induce ulcer model)	Methanolic extract	300 mg/kg	5.0 \pm 0.4 ^a	0.5 \pm 0.0 ^a	67 \pm 3.1 ^a
	Methanolic extract	500 mg/kg	4.5 \pm 0.5 ^a	0.4 \pm 0.1 ^a	73 \pm 3.2 ^a
	Methanolic extract	700 mg/kg	2.7 \pm 0.4 ^a	0.3 \pm 0.0 ^a	80 \pm 2.6 ^a
	<i>n</i> -Hexane extract	300 mg/kg	4.6 \pm 0.3 ^a	0.5 \pm 0.0 ^a	67 \pm 2.3 ^a
	<i>n</i> -Hexane extract	500 mg/kg	4.2 \pm 0.3 ^a	0.4 \pm 0.0 ^a	75 \pm 2.0 ^a
	<i>n</i> -Hexane extract	700 mg/kg	2.5 \pm 0.4 ^a	0.2 \pm 0.0 ^a	80 \pm 2.2 ^a
	Decoction	10 mL/kg	5.6 \pm 0.5 ^a	0.6 \pm 0.1 ^a	60 \pm 3.9 ^a

Data represented as mean \pm SEM; ^a $p < 0.05$ when compared with disease control; ns = non-significant when compared with disease control

methanolic and *n*-hexane extracts of *C. nocturnum* leaves had shown significant anti-ulcer activity in a dose-dependent manner (Figure 1).

Discussion

Treatment groups in both ulcer models showed significant ($p < 0.05$) anti-ulcer effect in methanol- and *n*-hexane-treated groups which is comparable to the effect of standard drug while decoction showed less protection of mucosal and epithelial cells against corrosive action of ethanol and indomethacin in stomach.

Histopathological analysis of gastric mucosa of treated rats showed dose-dependent anti-ulcer effect of both extracts whereas decoction could not protect gastric mucosa to the level that extracts did. This might be due to chemical constituents that were extracted in methanol and *n*-hexane extracts.

Banerjee and Firdous, (2015) studied *Ipomoea staphylina* hydroalcoholic extract for anti-ulcer activity to find out

new anti-ulcer drug with less adverse effects as compared to currently available ulcer treatment medicines. This plant extract showed significant decrease in free acidity, total acidity, and ulcer index which was supported by histopathological analysis. Current results are in agreement with Banerjee and Firdous findings (Banerjee and Firdous; 2015). Gul et al., (2015) explored anti-ulcer activity of *Menthe longifolia* and found decline in ulcer index (Gul et al., 2015). *Dalbergia sissoo* has been discovered to possess anti-ulcer activity by gastric mucosal protection (Gul et al., 2016). Our results are in accordance with the findings of studies conducted by Gul et al., 2015 and 2016.

Increased gastric juice secretion and decreased gastric pH lead to increase in acidity in stomach which if not treated at early stage may cause gastric ulceration. *C. nocturnum* leaves extracts showed anti-ulcer activity by decreasing lesions score, reducing gastric juice volume and acidity and increasing gastric pH.

Table II

Effect of *Cestrum nocturnum* extracts on gastric juice pH, volume and acidity

Group	Treatment	Dose	pH of gastric juice	Volume of gastric juice	Free acidity (mEq/L)	Total acidity (mEq/L)
Group I (Normal control)	Normal saline	2 mL/kg	2.0 ± 0.1 ^{ns}	3.5 ± 0.6 ^a	40.5 ± 2.9 ^a	51.4 ± 4.5 ^a
Group II (Disease control)	Ethanol	1 mL/kg	1.1 ± 0.1	4.3 ± 0.1	59.5 ± 2.1	68.4 ± 3.5
	Indomethacin	30 mg/kg	1.9 ± 0.1	4.1 ± 0.7	55.1 ± 1.7	61.4 ± 5.7
Group III (Standard)	Omeprazole	20 mg/kg	4.7 ± 0.2 ^a	2.0 ± 0.1 ^a	13.8 ± 3.1 ^a	28.2 ± 4.6 ^a
Group IV (Ethanol-induced ulcer model)	Methanolic extract	300 mg/kg	3.6 ± 0.1 ^a	2.5 ± 0.1 ^a	18.8 ± 1.1 ^a	38.5 ± 1.3 ^a
	Methanolic extract	500 mg/kg	3.9 ± 0.1 ^a	2.3 ± 0.1 ^a	15.8 ± 0.6 ^a	32.6 ± 1.4 ^a
	Methanolic extract	700 mg/kg	4.9 ± 0.3 ^a	2.6 ± 0.1 ^a	12.8 ± 1.3 ^a	25.1 ± 2.8 ^a
	<i>n</i> -Hexane extract	300 mg/kg	3.8 ± 0.2 ^a	2.3 ± 0.1 ^a	17.2 ± 1.1 ^a	35.9 ± 1.6 ^a
	<i>n</i> -Hexane extract	500 mg/kg	4.4 ± 0.3 ^a	2.4 ± 0.1 ^a	15.1 ± 1.0 ^a	33.9 ± 2.4 ^a
	<i>n</i> -Hexane extract	700 mg/kg	5.4 ± 0.2 ^a	2.1 ± 0.1 ^a	10.5 ± 0.8 ^a	23.0 ± 2.9 ^a
	Decoction	10 mL/kg	2.5 ± 0.1 ^a	3.4 ± 0.1 ^a	25.5 ± 0.7 ^a	45.1 ± 1.5 ^a
Group V (Indomethacin-induced ulcer model)	Methanolic extract	300 mg/kg	3.5 ± 0.2 ^a	2.8 ± 0.1 ^a	18.2 ± 1.4 ^a	38.4 ± 3.2 ^a
	Methanolic extract	500 mg/kg	3.5 ± 0.1 ^a	2.6 ± 0.1 ^a	17.2 ± 1.5 ^a	35.9 ± 3.6 ^a
	Methanolic extract	700 mg/kg	5.3 ± 0.3 ^a	2.4 ± 0.1 ^a	10.9 ± 1.1 ^a	23.0 ± 2.1 ^a
	<i>n</i> -Hexane extract	300 mg/kg	3.5 ± 0.2 ^a	2.4 ± 0.1 ^a	17.9 ± 1.3 ^a	37.1 ± 3.4 ^a
	<i>n</i> -Hexane extract	500 mg/kg	4.0 ± 0.2 ^a	2.6 ± 0.1 ^a	15.4 ± 0.5 ^a	31.7 ± 0.8 ^a
	<i>n</i> -Hexane extract	700 mg/kg	5.3 ± 0.3 ^a	2.6 ± 0.1 ^a	9.8 ± 1.9 ^a	23.4 ± 2.1 ^a
	Decoction	10 mL/kg	3.7 ± 0.1 ^a	3.0 ± 0.04 ^a	2.0 ± 1.0 ^a	38.8 ± 1.4 ^a

Data were represented as mean ± SEM; * $p < 0.05$ when compared with disease control; ns = non-significant when compared with disease control

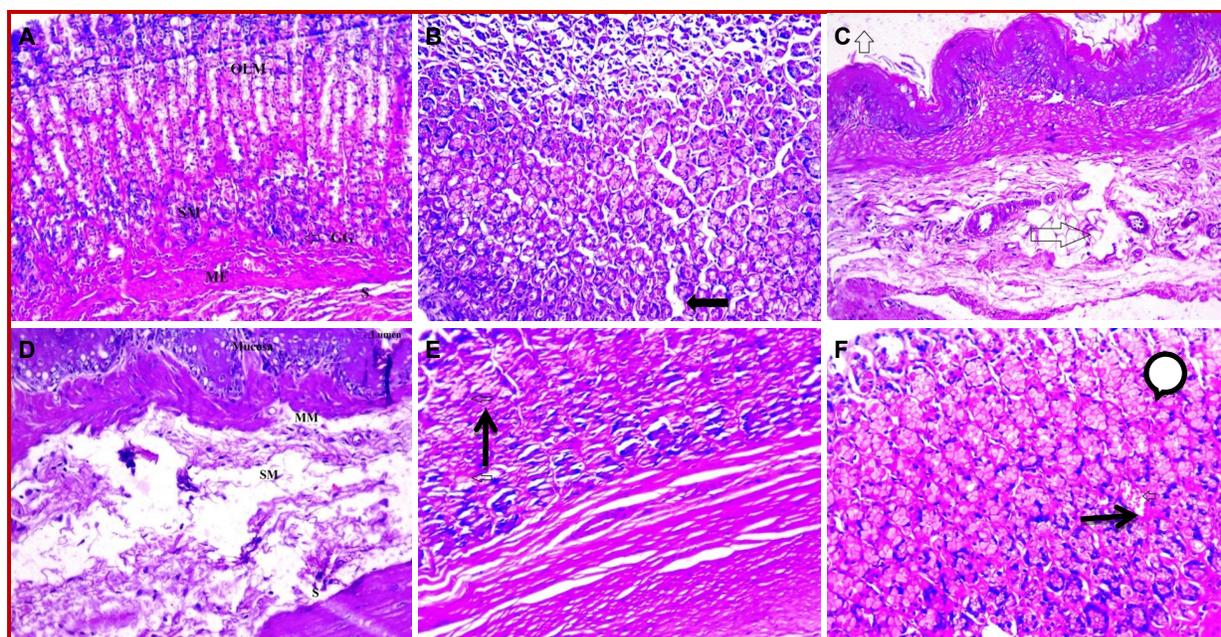


Figure 1: Histological examination of gastric ulcers; A: Photomicrograph of rat stomach section from the control group showing the histological structure of the normal submucosa (SM), gastric gland (GG), serosa (S), muscularis externa (ME), outer longitudinal muscle (OLM); B: Standard group (omeprazole) is showing normal appearance of Gland cell and sub mucosal cell; C: Disease control (Ethanol induce ulcer) is indicating mucosal and sub mucosal ulceration along with loss epithelium cells in gastric mucosa; D: Disease control group (Indomethacin) is showing stomach of the ulcer control animal showing a severe effect on the submucosa with erosion, edema, moderate leucocyte infiltration and cellular debris; E: Methanolic extract 750 mg/kg + ethanol treated group is showing normal muscularis mucosa (MM), Submucosa (SM) and blood in mucosa cells; F: *n*-hexane 750 mg + ethanol treated group is indicating condensation of mucosa and clustering of RBC's in sub mucosal layer

Conclusion

Methanolic and *n*-hexane extracts of *C. nocturnum* leaves have anti-ulcer and anti-secretory activity against ethanol and indomethacin induced ulcer models.

Conflict of Interest

All authors have completed the ICMJE uniform disclosure form and declare no support from any organization for the submitted work.

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