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Research Article

ANTI-ULCEROGENIC EFFECTS OF *LANTANA CAMARA* LINN. LEAVES ON *IN VIVO* TEST MODELS IN RATS

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ABSTRACT

Ethnobotanical knowledge of medicinal plants is some of the most prominent sources of new drugs and has shown potential results for the treatment of gastrointestinal disorders. In order to establish the pharmacological basis for the ethno medicinal use of *Lantana camara* Linn. in gastrointestinal affections, this study examined the effects of methanol extract of leaves (MELC), on aspirin, ethanol and cold restraint stress induced gastric lesions in rats. Study has shown anti-ulcer activity of extract in dose dependent manner. MELC decreases volume of gastric juice, total acidity, free acidity and increases pH significantly ($P<0.001$) in aspirin induced gastric ulcer. Pre-treatment with the extract (200 and 400 mg/kg) showed ulcer protective effect in aspirin induced (63.31%, 71.02% protection), ethanol induced (85.79%, 93.09% protection) and cold restraint stress induced (46.86%, 63.90% protection) ulcer models. Extract also possess *in vivo* antioxidant activity as it increases superoxide dismutase, catalase, reduces glutathione in extract treated group. The results indicate anti-ulcer potential of MELC

Key words: *Lantana camara*, methanol extract, anti-ulcer, antioxidant.

INTRODUCTION

Peptic ulcer and gastric hyperacidity are very common and one of the major gastro-intestinal disorders causing human suffering today. Peptic ulcer occurs mainly due to imbalance between mucosal defensive factors such as bicarbonate, prostaglandin, nitric oxide, peptides, growth factors and injurious factors like acid, pepsin [1]. Gastric ulcer is often a chronic disease and may continue for 10-12 years characterized by recurring episode of healing and re-excerbations [2]. Anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are also known to results severe gastric irritation [3]. Free radicals have been implicated in the pathogenesis of peptic ulcer and a wide variety of clinical disorders and physical, chemical and psychological factors also contribute in this regards [4]. Therefore reduction of gastric acid production as well as protection of gastric mucosa has been the major approaches for treatment of peptic ulcer [5].

Lantana camara Linn. is a low, erect or scandent shrub, grow upto 1.2-2.4 m high, with stout recurved prickles and having strong odour of black currants, introduced into India as an ornamental plant but now entirely naturalized and found throughout India. Leaves are opposite, ovate or ovate-oblong, acute or subacute, crenate-serrate, rugose above, scabrid on both side [6]. The traditional use of the plant mainly refer to its antiinflammatory, emmenagogue, antitussive, antimalarial, carminative, antispasmodic, antipyretic, anti-ulcer and wound healing property [7, 8, 9].

The lack of potent anti-ulcer drugs with less side effects in use prompted us for the present study. However so far no systematic study on anti-ulcer activity has been reported in the literature. The present study was attempted to investigate the traditional claim of *Lantana camara* Linn. for anti-ulcer activity.

MATERIALS AND METHODS

Plant materials

The leaves of *Lantana camara* were collected in October 2009 from Komarapalayam, Tamil Nadu and were authenticated by Botanical Survey of India (BSI), Ministry of Environment and Forests, Government of India, Coimbatore, Tamil Nadu (No.BSI/SC/5/23/08-09/Tech.1781). A voucher specimen was deposited in Department of Phytopharmacy and Phytomedicine, J.K.K. Munirajah Medical Research Foundation, College of Pharmacy, Komarapalayam, Tamil Nadu, India.

Preparation of extract

The leaves of *Lantana camara* were dried under shade and then made into a coarse powder. Air dried powdered material (500 gm) was first extracted with petroleum ether for 18 hrs using soxhlet apparatus. Marc was dried and extracted again with methanol for 18 hrs till the solvent become colourless. Extract obtained was concentrated in vacuum under reduced pressure using rotary flask evaporator. It was further concentrated and dried in the dessicator for further studies.

Preliminary phytochemical investigation

The methanol extract was subjected to qualitative chemical test for the identification of different phytoconstituents like sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins, triterpenoids [9, 10].

Test animals

Wistar albino rats of either sex weighing between 150-250 g (6–8 weeks old) were used for the study. They were kept in the departmental animal house at 25±2°C and relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 18–24 h before the experiment though water was allowed *ad libitum*. All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee was obtained as per the prescribed guidelines.

Aspirin induced ulcers

The experiment was performed according to the method of Kannappan (2008) [11]. Animals were divided into five groups each consist of six animals. Group I served as normal control; group II served as ulcer control and treated with 1% CMC. Group III and IV treated with methanol extract of *Lantana camara* leaves (MELC) at a dose of 200 and 400 mg/kg, p.o. respectively and standard drug ranitidine (20 mg/kg, p.o.) administered to group V. In this model, gastric lesions were induced by aspirin (200 mg/kg, p.o.) administered to rats of group II-V after 1 hr of respective drug treatment. The animals were sacrificed by cervical dislocation after 4 hrs of aspirin administration for the determination of ulcerative lesion index, gastric juice volume and acidity.

Cold restraint stress induced ulcers

Animals were divided into 5 groups and subjected to drug treatment as described above, omeprazole (20 mg/kg) used as standard drug. One hour after the drug treatment, the rats were immobilized by strapping the limbs and kept for 2 hrs at temperature of 3-5°C for three consecutive days. The animals were fasted for 24 hrs on final day in steel cages to avoid corophagy and the animals were killed by cervical dislocation and ulcers were examined on the dissected stomach after induction of stress [12].

Ethanol induced ulcers

The gastric ulcers were induced in rats by administrating 100% ethanol at a dose of 5 ml/kg of body weight, orally after 1 hr of MELC (200 and 400 mg/kg, p.o.) and sucralfate (100 mg/kg, p.o.) treatment to all groups of animals. Animals were sacrificed after 1 hr of ethanol treatment and stomach was incised along the greater curvature and examined for ulcers [13].

Measurement of gastric secretion and pH

The stomach of aspirin induced ulcer rats was carefully excised keeping oesophagus closed and opened along greater curvature and luminal contents were removed. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min and expressed in terms of ml/100 g of body weight. The pH of the supernatant was measured using digital pH meter [14].

Free and total acidity were determined by titrating with 0.01N NaOH using Topfer's reagent and phenolphthalein respectively as indicators and were expressed as meq/l per 100 g [15].

Measurement of ulcer index

Stomach mucosa was flushed with saline and lesions in glandular portion were then exposed and examined under a 10x magnifying glass [16]. Ulcer index of each animal was calculated by adding the values and their mean values were determined by the following scoring system (Malairajan et al., 2007) (i) Normal coloured stomach – 0, (ii) red colouration - 0.5, (iii) spot ulceration – 1, (iv) haemorrhagic streak – 1.5, (v) ulcers – 2, (vi) perforations – 3.

Percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{UI_{\text{ulcer control}} - UI_{\text{treated}}}{UI_{\text{ulcer control}}} \times 100$$

Assay of antioxidant enzymes

Gastric mucosa was scraped from the glandular part of the stomach, suspended in 5.0 ml of cooled 0.15 M Kcl-10mM potassium phosphate buffer (pH 7.4) containing 0.1% Triton X -100 and centrifuged at 1000 rpm for 10 min [17]. This tissue homogenate was used for the estimation of superoxide dismutase (SOD) [18], catalase (CAT) [19] and reduced glutathione (GSH) [20].

Statistical analysis

All the values are expressed as mean ± S.E.M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison test. The values are statistically significant at three levels, ***P<0.001, **P<0.01, *P<0.05.

RESULTS

Preliminary phytochemical analysis

The preliminary phytochemical screening of the extract of *Lantana camara* leaves showed the presence of carbohydrates, glycosides, sterols, flavonoids, saponins, tannins and phenolic compounds.

Effect of *Lantana camara* on gastric secretion and pH in aspirin induced ulcer

Pretreatment with methanol extract of *Lantana camara* leaves produced significant anti-ulcer effect which can observed by the effect of MELC on gastric secretion in aspirin induced ulcer (Table 1). Gastric juice volume, total and free acidity significantly increased and pH decreased in ulcer control animal in comparison to normal animals. MELC (200 and 400 mg/kg) produced dose dependent effect and decreased gastric juice volume, total and free acidity and increased pH significantly.

Anti-ulcer effect of *Lantana camara*

Oral administration of methanol extract of *Lantana camara* leaves at a dose of 200 and 400 mg/kg exhibited dose dependent inhibition percentage of 63.31 and 71.02 respectively compared to the ulcer control, proving the anti-ulcer activity of extract whereas ranitidine (20 mg/kg) produced 88.77% inhibition of ulcer index against aspirin induced ulcer (Table 2). Table 3 represents the data of anti-ulcer activity of MELC on ethanol induced ulcer. MELC significantly protected gastric mucosa against the damage induced by ethanol and curative ratios of the MELC at a dose of 200 and 400 mg/kg were found to be 85.79 and 93.09% respectively. Oral administration of MELC 1 hr before the induction of stress reduced the cold restrained stress induced ulcers. The MELC exhibited a dose dependent inhibition percentage of 46.86 and 63.90 at doses of 200 and 400 mg/kg dose respectively. The standard drug omeprazole showed an inhibition percentage of 82.77. The results are tabulated in Table 4.

***In vivo* antioxidant effect of *Lantana camara* leaves extract**

In order to explore the effects of antioxidant defences on the process of ulceration, in all stomach tissues, the antioxidant levels were evaluated. The level of superoxide dimutase (SOD), catalase (CAT) and glutathione (GSH) were evaluated in normal, ulcer control and drug treated group. Methanol extracts of *Lantana camara* leaves showed a dose dependent antioxidant activity ($p<0.001$) in all three anti-ulcer models and results were shown in Tables 5, 6 and 7. Aspirin, ethanol and cold restraint stress produced depletion of enzymatic antioxidant like CAT and SOD, also decreased the level of GSH which is a non-enzymatic antioxidant. Pretreatment with MELC elevated the levels of CAT, SOD and GSH to normal in all three ulcer models.

Table 1: **Effect of *Lantana camara* on gastric juice volume, pH, total and free acidity in aspirin induced ulcer**

Groups	Gastric juice volume (ml/100 g)	pH	Total Acidity (meq/l/100 g)	Free Acidity (meq/l/100 g)
Normal Control	1.21 ±0.09	2.01 ±0.11	52.97 ±1.93	27.30 ±1.73
Ulcer Control	2.36 ±0.08***	1.36 ±0.12***	72.42 ±3.16***	41.35 ±1.66***
MELC (200 mg/kg)	1.90 ±0.05**	1.85 ±0.05**	60.56 ±1.06**	35.88± 2.14**
MELC (400 mg/kg)	1.75 ±0.07***	2.18 ±0.15***	51.84 ±1.19***	29.24 ±1.75***
Ranitidine (20 mg/kg)	1.11 ±0.09***	2.70 ±0.17***	47.72 ±1.39***	26.70 ±0.99***

All values are expressed as mean ± S.E.M; n=6 animals in each group. ***P<0.001, **P<0.01 when ulcer control group was compared with normal control group and drug, extract treated groups were compared with ulcer control group.

Table 2: Effect of methanol extract of *Lantana camara* leaves on aspirin induced ulcers

Groups	Ulcer index(ui)	Percentage inhibition
Normal Control	00.00 ± 0.00	-
Ulcer Control	28.16 ± 2.27***	-
MELC (200 mg/kg)	10.33 ± 0.91***	63.31
MELC (400 mg/kg)	8.16 ± 0.54***	71.02
Ranitidine(20 mg/kg)	3.16 ± 0.10***	88.77

All values are expressed as mean ± S.E.M; n=6 animals in each group. ***P<0.001, **P<0.01, ulcer control group was compared with normal control group and drug, extract treated groups were compared with ulcer control group.

Table 3: Effect of methanol extract of *Lantana camara* leaves on ethanol induced ulcers

Groups	Ulcer index(ui)	Percentage inhibition
Normal Control	00.00 ± 0.00	-
Ulcer Control	63.00 ± 1.93***	-
MELC (200 mg/kg)	8.95± 0.91***	85.79
MELC (400 mg/kg)	4.35 ± 0.55***	93.09
Sucralfate(100 mg/kg)	3.33 ± 0.91***	94.71

All values are expressed as mean ± S.E.M; n=6 animals in each group. ***P<0.001, **P<0.01, ulcer control group was compared with normal control group and drug, extract treated groups were compared with ulcer control group.

Table 4: Effect of methanol extract of *Lantana camara* leaves on cold restraint stress induced ulcers

Groups	Ulcer index(ui)	Percentage inhibition
Normal Control	00.00 ± 0.00	-
Ulcer Control	16.43 ± 2.27***	-
MELC (200 mg/kg)	8.73 ± 0.91**	46.86
MELC (400 mg/kg)	5.93 ± 0.55***	63.90
Omeprazole(20 mg/kg)	2.83 ± 0.10***	82.77

All values are expressed as mean ± S.E.M.; n=6 animals in each group. ***P<0.001, **P<0.01, ulcer control group was compared with normal control group and drug, extract treated groups were compared with ulcer control group.

Table 5: Effect of MELC on antioxidant levels in aspirin induced ulcer model

Groups	Cat (u/mg protein)	Sod (u/mg protein)	Gsh (u/mg protein)
Normal Control	97.21± 0.95	114.31 ± 0.37	5.36 ± 0.48
Ulcer Control	68.51±0.70***	87.61±0.24***	2.53±0.49***
MELC (200mg/kg)	79.28±0.47***	99.16±0.66***	4.03± 0.15***
MELC (400mg/kg)	87.97±0.61***	105.21±0.42***	4.13± 0.18***
Ranitidine (20mg/kg)	95.61±1.04***	111.12±0.51***	4.95 ± 0.17***

All values are expressed as mean ± S.E.M; n=6 animals in each group. ***P<0.001, **P<0.01, ulcer control group was compared with normal control group and drug, extract treated groups were compared with ulcer control group.

Table 6: Effect of MELC on CAT, SOD and GSH in ethanol induced ulcer model

Groups	Cat (u/mg protein)	Sod (u/mg protein)	Gsh (u/mg protein)
Normal Control	91.23± 0.32	106.49± 0.93	4.56 ± 0.12
Ulcer Control	53.03±0.40***	72.86± 0.92***	1.55± 0.08***
MELC(200 mg/kg)	70.58±0.53***	86.45± 0.53***	2.85± 0.14***
MELC(400 mg/kg)	78.89±0.66***	96.80± 0.98***	3.68± 0.07***
Ranitidine(20mg/kg)	89.14±0.69***	103.49± 0.53***	3.96± 0.05***

All values are expressed as mean ± S.E.M; n=6 animals in each group. ***P<0.001, **P<0.01, ulcer control group was compared with normal control group and drug, extract treated groups were compared with ulcer control group.

Table 7: Effect of MELC on CAT, SOD and GSH in cold restraint stress induced ulcer

Groups	Cat (u/mg protein)	Sod (u/mg protein)	Gsh (u/mg protein)
Normal Control	94.96 ± 0.75	110.66± 0.69	4.93 ± 0.53
Ulcer Control	66.58± 0.57***	89.47± 0.37***	2.28± 0.31***
MELC(200 mg/kg)	76.54± 0.77***	96.49± 0.40***	3.44± 0.29***
MELC(400 mg/kg)	84.83± 0.48***	103.43± 1.01***	3.68± 0.35***
Ranitidine(20 mg/kg)	93.56± 0.40***	107.44± 0.40***	4.76± 0.31***

All values are expressed as mean ± S.E.M; n=6 animals in each group. ***P<0.001, **P<0.01, ulcer control group was compared with normal control group and drug, extract treated groups were compared with ulcer control group.

DISCUSSION

Peptic ulcers are caused due to increase in gastric acid and/or decrease in gastric mucosal protection mechanisms. Potent anti-ulcerogenic and ulcer-healing drugs are act via decreasing offensive factors or of increasing the defensive factors [5]. In this work, we have studied anti-ulcerogenic activity of *Lantana camara* leaves in three different models including aspirin, ethanol and cold restraint stress induced ulcer, where ulcerogens produce ulcer is either due to the effect on acid secretion or on cytoprotection or both.

Aspirin is a commonly used non-steroidal anti-inflammatory drug (NSAIDs) and a potent cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogenous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow [12]. Aspirin increases acid secretion and produce microvasculature damage by generation of free radicals [21]. Treatment with MELC decreased volume of acid secretion, decrease total and free acidity, increased pH and decreased the ulcer index compared to ulcer control group, indicate the anti-ulcer potential of the extract. The pathogenesis of ethanol induced gastric damage in rats is complicated and involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leucotriene C4. These mediators act on gastric microvasculature, triggering a series of events that lead to mucosal and sub mucosal damage [22]. MELC significantly decreased the ulcer index may be due to its cytoprotective mechanism.

Cold restrained stress provides both emotional stress as well as physiological stress to the animal. Omeprazole was used here to study the proton pump inhibitor mechanism. Cold restrained stress induced ulcers are result of autodigestion of gastric mucosal barrier, accumulation of HCl and generation of free radicals [5, 12]. MELC showed a dose dependent ulcer curative ratio in cold restrained stress induced ulcers. The ulcer inhibition percentage of extracts was not closer to the standard drug omeprazole, but the extract significantly scavenged free radicals. Therefore, it may be concluded that MELC may not follow the proton pump inhibitory mechanism.

Previous investigation showed that administration of NSAIDs, ethanol and stress decreased both enzymatic and non enzymatic antioxidant levels and produced oxidative stress [23]. Oxidative stress plays an important role in the pathogenesis of ulcers. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism. The damage to the membrane proteins decreases membrane permeability, activities of enzyme and receptors and activation of cells [24]. Similarly in the present study the levels of SOD, CAT and GSH were decreased by administration of aspirin, ethanol and by induction of stress. Whereas, the administration of *L. camara* extract resulted in a significant increase in the SOD, CAT and GSH levels. SOD is an important endogenous antioxidant enzyme that acts as the first line defense system against ROS which scavenges superoxide radicals. SOD catalyzes superoxide to H_2O_2 and O_2 while CAT converts H_2O_2 to water and molecular oxygen preventing the oxidative damage. Glutathione (GSH) is a tripeptide and a powerful antioxidant present within the cytosol of cells and is the major intracellular non protein thiol compound (NPSH). GSH is important in maintaining –SH groups in other molecules including proteins, regulating thiol-disulfide status of the cell, and detoxifying foreign compounds and free radicals [24, 25].Thus the ability of the *Lantana camara* extract to scavenge the free radicals may contribute to the gastric cytoprotective activity.

Hence, our present study explores the anti-ulcer potential of methanol extract of *Lantana camara* leaves. The investigation on mode of action may pave way for establishment of new anti-ulcer therapy regimen.

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